Universidade de Aveiro Departamento de Biologia
Ano 2015

JULIANA RODRIGUES GADELHA

RESPOSTAS AO STRESS DE ANÉMONAS DO MAR EM TRÊS CENÁRIOS CLIMÁTICOS COMO DETECTORES PRECOCES DE ALTERAÇÕES AMBIENTAIS

SEA ANEMONES STRESS RESPONSES IN THREE DIFFERENT CLIMATIC SCENARIOS AS EARLY WARNING SYSTEMS FOR ENVIRONMENTAL CHANGES

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“Só existe um lugar onde o sucesso vem antes do trabalho, é no dicionário” (Albert Einstein).

o júri

presidente

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palavras-chave

Caracterização Ambiental, Estressores Químicos, Estresse Oxidativo,Bioacumulação, Anémonas do Mar, Cenários Climáticos, Detectores Precoces.

Resumo

A Caracterização de ambiente costeiro é um passo importante na compreensão dos processos químicos e físicos. Propõe-se um modelo de abordagem de ampla caracterização (grandes áreas geográficas, três zonas climáticas distintas) de estresses ambientais (temperatura-relacionadas), o que permite aos pesquisadores analisar seus estudos experimentais em relação à população geral de ambientes que eles são alvo. Para os padrões de qualidade da água, foram investigadas amostras ambientais e realizadas determinações dos parâmetros físicoquímicos e dos processos biogeoquímicos. Esta classificação ambiental poderia ser aplicada para futuros estudos com populações naturais e simulações de estresse ambiental e/ou testes laboratoriais com estressores químicos e físicos. Para entender melhor o estado da arte, foram escolhidos sete locais de amostragem, a fim de verificar em diferentes zonas climáticas, os padrões de resposta de populações naturais a estressores físicos e químicos. Na União Europeia (UE) cerca de 3000 substâncias diferentes são usadas na medicina humana, como analgésicos e anti-inflamatórios, anticoncepcionais, antibióticos, beta-bloqueadores, os reguladores lipídicos, compostos neuroativos e muitos outros estressores ambientais (químicos). Nos últimos anos, o conhecimento sobre a ocorrência no meio ambiente marinho e costeiro de produtos farmacêuticos e outros poluentes tem aumentado em grande medida devido às novas técnicas analíticas capazes de determinar compostos polares em pequenas quantidades. A avaliação dos valores de bioacumulação em misturas ambientais complexas requer a aplicação de procedimentos de integração, combinando análises químicas e ensaios biológicos específicos.

Esta abordagem foi focada na saúde e compostos ambientalmente relevantes para com base na avaliação de bioacumulação, encontrar uma correlação entre as concentrações dos compostos organoclorados, congêneres de chlorobiphenil e hidrocarbonetos policíclicos aromáticos em amostras de sedimento e espécies de anêmonas co-genéricas em três cenários climáticos diferentes. As anêmonas do mar das espécies *Anemonia sargassensis, Anemonia sulcata, Actinia bermudensis, Actinia equina, Bunodossoma caissarum* e *Bunodossoma canjicum* foram escolhidas porque podem, eventualmente, ser boas indicadores de poluição e sinalizadoras de alerta precoce. Após a identificação do estado do meio ambiente com os dados abióticos, fatores físico-químicos, estudos caracterizações químicas e em como as espécies foram afetadas no estado de "doença" ambiental, na avaliação da contaminação química. Finalmente foi possível aplicar as ferramentas para detectar como e o quanto esses estressores químicos e / ou físicos podem afetar os organismos fisiológica, bioquímica, anatomicamente e alterações estruturais. As ferramentas mais utilizadas para avaliação de estressores de perigo químico consiste nos biomarcadores enzimáticos. Biomarcadores têm sido amplamente utilizados para a avaliação da exposição e / ou efeitos para os contaminantes ambientais. Os biomarcadores de estresse oxidativo é comumente utilizado em organismos marinhos para avaliar os níveis de estresse e os efeitos e danos causados pelos diferentes estressores naturais, físicos e/ou químicos. O principal objetivo deste estudo é detectar significativos níveis basais de atividade da GST, GR, CAT, LPO e SOD mensurável como biomarcadores de estresse ambiental, em populações naturais de anêmonas do mar, sob influência de diferentes fontes de poluição em três cenários climáticos. Realizando assim, a validação dos níveis de atividade enzimática e avaliando as variações espaciais e interespecíficas.

keywords

Environmental Characterization, Chemical Stressors, Oxidative Stress, Bioaccumulation, Sea Anemones, Climatic Scenarios, Early Warning.

Abstract

Characterization of the coastal environment is an important step to understand the chemical and physical processes. A modelling approach is proposed here to characterize broadly (large geographic area, three distinct climatic zones) temperature-related environmental stresses, which enables breeders to analyse their experimental trials with regard to the broad population of environments who they target. Water quality patterns experienced environmental samplings were determined for marine physicochemical and biogeochemical processes. This environment classification applied to future environmental natural populations stress simulations and chemical and physical stress laboratorial simulations. Understanding the state of the art, it chosen a seven sampling sites in order to verify on different climatic zones, natural populations patterns to answer to chemical and physical stressors. In the European Union (EU) about 3000 different substances used in human medicine such as analgesics and anti-inflammatory drugs, contraceptives, antibiotics, beta-blockers, lipid regulators, neuroactive compounds and many others (chemical environmental stressors). In the last few years, knowledge about the marine and coastal environmental occurrence of pharmaceuticals and other pollutants has increased largely due to new analytical techniques able to determine polar compounds at trace quantities. The assessment of bioaccumulations values in complex environmental mixtures requires application of integrative procedures combining chemical analysis and specific bioassays.

This approach focused on health and environmentally relevant compounds and based on bioaccumulation evaluation, to find a correlation between organochlorinated compounds, chlorobiophenyls congener and polycyclic aromatic hidrocarbons concentrations in sediments and congener sea anemones from three different climatic scenarios.

The sea anemones species *Anemonia sargassensis, Anemonia sulcata, Actinia bermudensis, Actinia equina, Bunodossoma caissarum* and *Bunodossoma canjicum* chosen because they might possibly be effective pollution indicators and early warning signilizer. After identification the environmental state with abiotic factors data, physico-chemical and chemical characterizations studies, how the species contribute to the environment sickness, evaluating the chemical pollutions bioconcentration; finally it was possible to apply tools to detect how and how much these natural, chemical and/or physical stressors could be affected the physiological, biochemical, anatomical and structural alterations. The most common tools to hazard chemical stressors consist on enzymatic biomarkers evaluation. Biomarkers have been widely used for the assessment of exposure and/or effects to environmental contaminants. The oxidative stress biomarkers is commom used on marine organism to assess the stress levels and effects and damages caused by natural, chemical and/or physical stressors. The main aim of this study is to detect significant basal levels activity GST, GR, CAT, LPO and SOD measurable as a biomarker environment, on natural populations of sea anemones, under environmental stress and sources of pollution in three different climatic scenarios. In order to detect different levels of contamination in the sampling locations, validating the combination of the activity levels of these enzymes with different pollutants sources and verify the spatial and interspecific variations.

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General Discussion

CHAPTER 1

General Introduction

Chapter 1. General Introduction

1.1. Environmental and Climatic Changes

At a time of global changes, the world is striving to face and adapt to inevitable, possible profound, alteration. Widening of droughts in southern Europe and sub-Saharan Africa, an increasing number of disasters, severe and more frequent flooding that could imperil low-lying islands, the crowded river deltas of southern Asia, are already taking place, and climate change will cause additional environmental stresses and societal crises in regions already vulnerable to natural hazards, poverty and conflicts. A global multi-hazard early warning system need to inform us of pending threats. This work try to find a databasis using natural population, supported with laboratorial experiments, in order to answers a some environmental stressors to early warning to global and environmental changes. It identifies current gaps and needs with the goal of laying out guidelines for developing a global multi-hazard early warning system. Many surface water bodies are now contaminated due to the increasing usage of pesticides, mainly in agriculture, petroleum derivated (polycyclic aromatic compounds) and to heavy metal contaminations from industry and/or natural sources and other sources pollution (Harbour, Antropogenic, Petrochemical, etc). This contamination may cause impairment of ecological functions (Fleeger et al., 2003) and decline of non-target species (Rohr et al., 2006).

1.1.1. Early warning to Environmental Systems

Early warning (EW) is “the provision of timely and effective information, through identified institutions, that allows individuals exposed to hazard to take action to avoid or reduce their risk and prepare for effective response”, and is the integration of four main elements according to the ‘United Nations’ International Strategy for Disaster Reduction (ISDR), it integrates (UN 2006):

1. *Risk Knowledge*: Risk assessment provides essential information to set priorities for mitigation and prevention strategies and designing early warning systems.

2. *Monitoring and Predicting:* Systems with monitoring and predicting capabilities provide timely estimates of the potential risk faced by communities, economies and the environment.

3. *Disseminating Information:* Communication systems are needed for delivering warning messages to the potentially affected locations to alert local and regional governmental agencies. The messages need to be reliable, synthetic and simple to be understood by authorities and the public.

4. *Response*: Coordination, good governance and appropriate action plans are key points in effective early warning. Likewise, public awareness and education are critical aspects of disaster mitigation (Moryaty, 1993).

Failure of any part of the system will imply failure of the whole system. For example, accurate warnings will have no impact if the population is not prepared or if the alerts are received but not disseminated by the agencies receiving the messages.

The basic idea behind early warning is that the earlier and more accurately we are able to predict short- and long-term potential risks associated with natural and human induced hazards, the more likely we will be able to manage and mitigate a disaster's impact on society, economies, and environment (tab. 1 and Fig. 1).

For this, the tools involved was ecotoxicology predictings. Ecotoxicology is interested in studying the effects of toxicants on the ecosystems. Pollutants matter because of their effects on populations and communities, through their effects on individual organisms (Moriarty, 1993). Since the immediate effects of pollutants are on organisms, either indirect (through habitat alterations) or direct (toxic effects of chemicals at the organismal level), one needs to assess what happens at the individual level to understand the impact on populations. The direct effect on individuals may range from rapid death through sub-lethal effects to no effects at all (Moriarty and Bell, 1993).

Ecotoxicology tests are needed to anticipate how toxicants are likely to impact ecological systems and to assess what changes are taking place in these systems under the influence of released toxic substances (Calow, 1997). When assessing the effects of a certain pollutant on a test species, the endpoints generally used are mortality (quantal type of data), and sub-lethal parameters like growth, reproduction, bioaccumulation and/or biomarker expression (continuous data), among others (Adams and Rowland, 2003). These responses can be ecologically relevant, as they are important components of fitness and determine the health, structure and dynamics of populations (Sibley et al., 1997).

1.1.2. Climatic Scenarios

For the ecotoxicological studies, exist many "standard environmental studies". The present study it was planned to give the enough information about the three diferent ecosystems states, in order to validate the use of sea anemomes as early warning to environmental and climate changes. Each climatic scenarios studied, present an individual characteristcs and we can find differences on natural populations to environmental stimuli and alterations. This alteration could be reflect on physical or chemical aspects (Chapter 3 and 4). The tropical, subtropical and temperate scenarios, naturally present an aspect in common, the temperature variation between them. These physical parameter, is responsible to alterations on the other parameters (salinity, conductivity, dissolved oxygen, nutrients, etc), including the "chemical parameters". Between these three types of ecosystems, the temperature is the major responsible for the environmental answer to global alterations.

The tropical scenarios is characterized by a climate typically found within the Tropics, while a few locations outside of the Tropics are considered to have a tropical climate. In the Köppen climate classification it is a non-arid climate in which all twelve months have mean temperatures of at least 18 °C (64 °F). Unlike the extra-tropics, where there are strong variations in day length and temperature, with season, tropical temperature remains relatively constant throughout the year and seasonal variations are dominated by precipitation.

Intertropical Convergence zone

Because of the effect of sun angle on climate most areas within the tropics are hot year-round, with diurnal variations in temperature exceeding seasonal variations. Seasonal variations in tropical climate are dominated by changes in precipitation, which are in turn largely influenced by the tropical rain belt or Intertropical Convergence Zone (ITCZ), a portion of the Hadley cell. The ITCZ is shown, for July average. Areas of ascending air have heavy rainfall; areas of descending air are dry. The ITCZ somewhat follows the solar equator throughout the year, but with geographical variations, and in some areas (India) is heavily influenced by local large-scale monsoons (Lineback and Gritzner, 2008).

The subtropical scenarios is characterized by a geographic and climate zones located roughly between the tropic circle of latitude (the Tropic of Cancer and Tropic of Capricorn) and the 38th parallel in each hemisphere. Subtropical climate regimes can exist at high elevations within the tropics, such as across the Mexican Plateau and in Vietnam and Taiwan. Six climate classifications utilize the term to help define the various temperature and precipitation regimes for the planet Earth. Eight months of the year within the subtropics have an average temperature at or above 10 °C (50.0 °F), with their coldest month averaging between 2 and 13 °C (35.6 and 55.4 °F) (Lineback and Gritzner, 2012).

A great portion of the world's deserts are located within the subtropics, due to the development of the subtropical ridge. Within savanna regimes in the subtropics, a wet season is seen annually during the summer, which is when most of the yearly rainfall falls. Within Mediterranean climate regimes, the wet season occurs during the winter. Areas bordering warm oceans are prone to locally heavy rainfall from tropical cyclones, which can contribute a significant percentage of the annual rainfall.

According to the E. Neef climate classification, the subtropical zone is divided into two parts: *Rainy winters of the west sides* and *Eastern subtropical climate*. According to the Wilhelm Lauer & Peter Frankenberg climate classification, the subtropical zone is divided into three parts: high-continental, continental, and maritime. According to the Siegmund/Frankenberg climate classification, subtropical is one of six climate zones in the world (Lineback and Gritzner, 2012).

The temperate scenarios is a geography, temperate or tepid latitudes of Earth lie between the tropics and the polar regions. The temperatures in these regions are generally relatively moderate, rather than extremely hot or cold, and the changes between summer and winter are also usually moderate (Mcknight and Darrel, 2000).

However, in certain areas, such as Asia and central North America, the variations between summer and winter can be extreme because these areas are far away from the sea, causing them to have a continental climate. In regions traditionally considered tropical, localities at high altitudes (e.g. parts of the Andes) may have a temperate climate (McColl, 2012).

The north temperate zone extends from the Tropic of Cancer (approximately 23.5° north latitude) to the Arctic Circle(approximately 66.5° north latitude). The south temperate zone extends from the Tropic of Capricorn(approximately 23.5° south latitude) to the Antarctic Circle (at approximately 66.5° south latitude). The cooler parts of the temperate zone may be referred to as 'subtemperate' (Brinch, 2007).

The maritime climate is affected by the oceans, which help to sustain somewhat stable temperatures throughout the year. In temperate zones the prevailing winds are from the west, thus the western edge of temperate continents most commonly experience this maritime climate. Such regions include Western Europe, and western North America at latitudes between 40° and 60° north (65°N in Europe) (Cohen, 1998).

Continental, semi-arid and arid are usually situated inland, with warmer summers and colder winters. Heat loss and reception are aided by extensive land mass. In North America, the Rocky Mountains act as a climate barrier to the maritime air blowing from the west, creating a semi-arid and continental climate to the east. In Europe, the maritime climate is able to stabilize inland temperature, because the major mountain range – the Alps – is oriented east-west (the area east of the long Scandinavian mountain range is an exception) (McColl, 2005; 2012; 2014).

The vast majority of the world's human population resides in temperate zones (if defined as comprising the subtropics as well), especially in the northern hemisphere because of its greater mass of land. The richest temperate flora in the world is found in southern Africa, where some 24,000 taxa (species and infraspecific taxa) have been described (Germishuizen and Meyer, 2003).

The samples sites selected for the present study it will appear detailed describe on each article. They was selected before a carefully biobliographical research, combined to a logistic planned between the research team. It was selected points in several points on the Atlantic: Northwestern Portuguese coast, Northest and Southest Brazilian coast and Southest Mexican Coast.

1.1.3. Target Species

The present study focused on sea anemones natural populations along the Atlantic coast. The species it was chosen after of a carefully bibliographical research, but even considerable the "cosmopolitan" characteristic of each species selected. Three genus it was chosen in order to appearance of one of them along the samples sites: *Actinia sp, Anemonia sp* and *Bunodossoma sp*.

Six target species were selected according their ecological importance, distribution and abundance. For the tropical environment it were selected *Anemonia sargassensis* (Hargitt, 1908) and *Bunodosoma cangicum* that are the most abundant sea anemones of Brazilian coast (Zamponi et al. 1998), with large distribution in medio and infralitoral habitats (Gomes and Mayal, 1997; Amaral et al. 2002). For the temperate environment it was selected

Actinia equina (Cornelius et al. 1995) and *Anemonia sulcata* (Cornelius et al. 1995) that are the most representative species found on rocky shores of the European coast and as far as the coast of West Africa (Its range extends throughout the Atlantic coasts of Europe, mainly in Iberian coast (Gadelha et al. 2010), North Africa and into the Mediterranean and South Africa (Stephenson, 1935). For the subtropical environment were selected two representative species, *Actinia bermudensis* and *Bunodosoma caissarum,* common on rocks just below the low tide line, that presents large distributions occurred since the West Indies, Bermuda and northern Florida and South to Brazil (Ruppert and Fox, 1988).

Actinia equina (Linnaeus, 1758)

The beadlet anemone, *Actinia equina*, is a common sea anemone found on rocky shores around all coasts of the United Kingdom. Its range extends to the rest of Western Europe and the Mediterranean Sea, and along the Atlantic coast of Africa as far south as South Africa. *Actinia equina* can be found both in exposed and sheltered situations. It is highly adapted to the intertidal zone as it can tolerate both high temperatures and desiccation. The anemone may also be found in regions of variable salinity such as estuaries.

Underwater, it displays up to 192 tentacles, arranged in six circles. Out of water, the tentacles retract and the anemone resembles a blob of red, brown, green or orange jelly, up to about 5 centimetres (2.0 in) across. It has bright blue beads (known as acrorhagi) located just beneath the tentacles, organised as an external ring containing stinging cells located at the top of the column that it uses to fight over territory. The acrorhagi contains the cnidocysts which themselves contain the nematocysts. There is some evidence that the various colour forms may in fact be different species.

Actinia equina is viviparous, with up to one hundred embryos developing inside the body cavity before being ejected into the open water as juveniles (Naylor, 2003) (Fig 2).

Actinia bermudensis (McMurrich, 1889)

Actinia bermudensis attaches itself to a rock surface by its pedal disc, which can reach 2.5 centimetres (1 in) in width. The column is narrower at the top than the base and can reach 5 centimetres (2 in) in height. Near the top is a ring of bulges called acrorhagi which contain many cnidocytes. The oral disc has a central mouth and two irregular whorls of 96 to 140 short, retractable, tapering tentacles which are armed with cnidocytes. The general colour of the anemone is dark red or maroon. In most of the range, the acrorhagi are blue, but in the waters off northern Florida, they are pink.

Actinia bermudensis occurs in the West Indies, Bermuda and northern Florida, and there is a further, isolated population off Brazil. It is found in the intertidal and the sublittoral zone. It is usually found near the base of rock walls, under overhangs, in caves, in crevices and under boulders (Monteiro et al., 1998) (Fig 2).

Anemonia sulcata (Pennant, 1777)

The snakelocks anemone (*Anemonia sulcata*) is a sea anemone found in the eastern Atlantic Ocean to the Mediterranean Sea. The tentacles of anemones in deep or murky water can be a grey colour, but are otherwise usually a deep green colour with purple tips due to the presence of symbiotic algae within the tentacles that use sunlight as an energy source. Since the anemones benefit from this. they prefer brightly lit shallow waters. On average the snakelock anemone is 8 cm wide (Morgado et al., 2008) (Fig 2).

Anemonia sargassensis, Hargitt, 1908 e *Bunodossoma caissarum*, Corrêa, 1964

The anemones *Bunodosoma caissarum* and *Anemonia sargassensis* are widely distributed along the Brazilian coast. The two species occupy rocky coasts, but in distinct distribution patterns: *B. caissarum* is intertidal and can be found exposed to the air during low tide, or trapped inside upper littoral tidal pools. On the other hand, *A. sargassensis* is subtidal and always found submerged, when in large tidal pools. The only physiological evaluation found in the literature for these species was one description of chemical transmission (Mendes, 1976) or of toxicity mechanisms (Malpezzi et al., 1993, Alés et al., 2000 and Oliveira et al., 2004), for *B. Caissarum. Bunodosoma caissarum*, a carnivorous marine species exclusive to the Brazilian southern coast, recognized as a sensitive bioindicator of artificial radioactive pollution, also shows a great capacity for concentrating natural α-emitters (Amado et al., 2011) (Fig 2).

Bunodossoma Cangicum, Belém and Preslercravo, 1973

The Anemone *Bunodosoma cangicum* Corrêa, 1964 is a species commonly found in mesolittoral between cracks and crevices in partial areas covered by pellet and fixed to the bedrock (Melo and Amaral, 2005). They very commom on northest beaches of Brazilian coast, under rocks and living in turves waters (Fig 2).

Figure 2. Six target species selected: *Actinia equina, Actinia bermudensis, Anemonia sulcate, Anemonia sargassensis, Bunodossoma cangicum* and *Bunodossoma caissarum.*

1.1.4. Effects of Climate Changes on Marine Ecosystem

In aquatic ecotoxicology, the effects of anthropogenic (and natural) toxicants on aquatic biota are studied. These contaminants enter (and can be deposited) in the aquatic environment either from direct discharge from effluents, terrestrial runoff, or atmospheric deposition. Biomonitoring of these effects may be done by routine monitoring (identify unanticipated contamination and effects) or by targeted monitoring (focused on specific, known contaminant situations) (Grue et al., 2002). In the aquatic environments, biomonitoring may involve sampling of organisms as an indication of possible contamination, and *in situ* tests by assessing acute and chronic toxicity in caged organisms exposed to either contaminated water, sediment or both. Laboratory toxicity tests are also an asset, either by transposing and assessing in the laboratory field organisms and/or contaminated water/sediment; by using test species cultured in the laboratory with field-contaminated water/sediment; or by assessing test species with artificially contaminated sediment/water. One must also bear in mind the ecological relevance of the experimental approach, in order to reach a compromise between realistic exposure situations and the scientific interest of the study.

Tested species can be representatives of the studied populations or model organisms that regularly used in toxicity tests, with well-studied endpoints. In this thesis the congeneer benthonic sea anemones adult polyps *Anemonia sargassensis, Anemonia sulcata, Actinia bermudensis, Actinia equina, Bunodossoma caissarum and Bunodossoma canjicum* used as model organisms. Non reports guidelines was used for this species, so these work was developed in order to standardize ecotoxicological tests and allow the replicability, repeatability and reproducibility of the experiments, thus increasing the test precision and uniformity among laboratories and for future comparision studies.

One of the drawbacks in using benthic macroinvertebrates for biomonitoring and assessment of water quality is the amount of effort required to process the samples, either in *in situ* tests (e.g. sorting animals in the sediment, measurements at the laboratory) or in laboratory tests where, in chronic tests, quantitative results on toxicity are only available at the end of the experimental period. For instance, to assess sub-lethal toxicity of pollutants on sea anemones in laboratory, results on the effects on survivor and reproduction are only available after several days [e.g. 96 hours and gonadal polyps still need to be measured (Gadelha *et al*, 2012) and to assess biomarker effects one needs to process the samples and quantify biomarker activities (Gadelha *et al*, 2015a; Domingues et al., 2007), which can be time consuming. Ecotoxicologists want to use bioassays that are quick and easy, giving valuable information readily on contaminant effects on individual organisms, in order to make predictions about long-term impacts at an ecological level. In fact, Forbes et al. (2006) referred the need to devote more effort in developing and improving methods that directly measure effects of chemical impacts on populations, communities and ecosystems, and that less effort must be invested on measures that, at best, can only ever be suggestive of risks.

Beitinger and McCauley (1990) suggested that responses to environmental changes can be divided in four categories: 1. passive – no response, when the stimulus is not sensed or occurs too rapidly thus leading to a decrease in performance capacities or even death; 2. behavioural reactions – when subjected to certain chemicals, animals usually react in seconds or minutes, avoiding stress and trying to obtain a favourable position relative to the level of stimulus; 3. physiological responses – organisms suffer internal changes in various physiological processes, including adjustments in physiological rate functions and tolerance acclimation enhancement, which may occur within hours to weeks; and 4. biochemical responses – synthesis of new molecules like ―stress‖ proteins in response to environmental change, in order to restore homeostasis within genetic constrains, which may take from days to weeks. So, adding behaviour as an endpoint can help to formulate a quantitative minute-to-minute or hour-to-hour assessment of how tested species are (re)acting towards the toxicant concentration, bearing in mind that behaviour can be classified as the cumulative interaction of a variety of biotic and abiotic factors that represents the animal's response to internal (physiological) and external (environmental, social) factors and that relates one organism to another (Dell'Omo, 2002). Behaviour provides an insight into various levels of biological organization, being a result and determinant of molecular, physiological, and ecological aspects of toxicology (Scott and Sloman, 2004). Therefore, behavioural responses may reflect biochemical changes in the individual organism and subsequently promote alterations in communities, which be translated into ecological consequences (Lagadic et al., 1994). Another endpoint was a bioconcentrations factors to chemical stressors, this endpoint could be provide a valiable information about the state of natural populations study (Gadelha *et al*, 2015a). One of environmental changes suggested categories it related to behavioural reaction. This category include when subjected to certain chemicals.

In former studies (e.g. with fish) behavioural parameters (considering swimming, ventilation, and foraging) have been suggested to be more sensitive than other endpoints (Beitinger, 1990; Beitinger and McCauley, 1990; Dell'Omo, 2002; Gerhardt, 2007). However, few studies have been made linking behavioural parameters to other biological (physiological, morphological and anatomic) and ecological responses.

The health of an aquatic ecosystem is degraded when the Ecosystem`s assimilative capacity to absorb a stress has been exceeded. A health ecosystem is composed of biotic communities and abiotic characteristics, with form a self- regulating and self-sustaining unit. Although changes within an ecosystem can result from naturally occurring events, anthropogenic activities often impose stresses on this system. When organisms of an ecosystem exposed to stress, their resistance to displacement from that ecosystems may exceeded. Depending upon the magnitude and temporal nature of the stress, the organisms may not be sufficiently resilient to reestablish their pre-stress community structure.

The community structure of aquatic ecosystems is sensitive to, and determine by, the conditions and resources available within a habitat. Conditions include abiotic factors environmental factors, which vary with time and space (e.g., temperature, salinity, and flow) (Begon et al., 1990 *in* Loeb and Spacie, 1994).

Resources are all things consumed by an organism (e.g., food, light, and space) (Tilman, 1982). Organisms that come to make up on aquatic community are those that can endure, tolerate, compete, reproduce, and persist within a given habitat. If a habitat characterized by condition that within acceptable limits and provides all necessary resources for a given species, that species could potentially occur there (Begon et al., 1990).

In essence, the above account define the nuche space of an organism, that is, an n-dimensional hypervolume of all ecological factores relati or categories of variableve to a species' ability to survive and multiply (Hutchinson, 1958). Hutchinson considered two general types of niche axes or categories of variables scenopoetic and binomic. Scenopoetic variables have no direct relashionship to competition; rather they are conditions that an organism must be able to tolerate. Bionomic variables involve resources for which there may be competition. If an aquatic organism is exposed to a stress that changes the conditions or resources of a habitat, the niche space whitin which an organism survives is changed and a new hypervolume with a response structure within which there is a point of optimal survival surrounded by areas of less than optimal survival (Maguire, 1973). A stress that leads to the alteration of any environmental characteristics of aquatic ecosystems would affect the survivorship of organisms living within that ecosystem. The result would be a restructuring of the biotic community that was originally present.

A stress on aquatic ecosystem can be categorize into one of three types: (1) physical; (2) chemical; or (3) biological alterations. Physical alterations include changes in water temperature, water flow, substrate/habitat type, and light availability. Chemical alterations include changes in the loading rayes of biostimulatory nutrients oxygen consuming materials, and toxins. Biological alterations include the introduction of exotic species. Activities that result in a change in any of these environmental characteristics can lead to the deformation of an organism's niche space, possibly leading to its extinction.

A decision must made concerning which attributes (i.e., variables) to measure when designing an assessment program to evaluate the health of an aquatic ecosystem. An attribute needs to be quantifiable such that the variance or uncertainty surrounding the measurement of that attribute can be determined. An attribute should be diagnostic in its ability to assist in the determination of whether an aquatic ecosystem is healthy or not. It also needs to be responsive to any change in resource availability and/or habitat conditions. A variety of attributes may be required adequately identify the transitional stages that a healthy aquatic ecosystem goes through as it suffers impairment and as a system recovers from an impaired state.

1.1.5. Chemical Stressors

Chemical variables have been the most commom attributes selected when designing environmental assessment programs of aquatic ecosystems. Chemical analysis are quantifiable and the variance surrounding any specific smapling episode is usually small. An understanting of the water chemistry can assist in an evaluaton of the type of stress that may exist (e.g., biostimulatory chemicals or toxins); however, diagnosis of the impact of these chemical substances on the aquatic ecosystem cannot be determined based solely on water chemistry. When used alone; the utility of water chemical analysis is limited. A static concentration of a chemical represents the residual of that element or compound after biological organisms have ingested, absorbed, stored or transformed it. Furthermore, abiotic materials (i.e., sediments) also modify the chemistry of waters through adsorption. Chemical analysis fail to account for the rates of transformation of a chemical by biological orgamisms and the possible relocation of a chemical within the aquatic ecosystem due to abiotic adsorption and sedimentation. Chemical attributes lack the responsiveness necessary to evaluate the health of an aquatic ecosystem. The dynamic interactions (physical, chemical, and biological) that characterize an ecosystem not adequately assessed through the sole use of chemical attributes.

The purpose of this section (related to the paper 1- chapter 2 and paper 3 chapter 4) is to provide an understanding of the efficacy of coastal monitoring assessing the environmental health of aquatic ecosystems.

The first step consisted to understanding the three climatic scenarios state, dynamic, physic chemical attributes and spatial variations to comprehend the natural conditions influences on organisms, namely sea anemones populations. Therefore, in this chapter we only discuss about environmental state to structure and conduct the follow chapters involving the biological components (see paper 1 chapter 2). After the environmental characterization, on the second chapter (paper 1), sampling sites, species and contaminant target chosen, we can introduce tools to investigate the bioaccumulations levels and chemical stress response. In this section, we separately investigate oil hydrocarbons (Paper 2), organic biocides, industrial pollutants (Paper 3). We will discuss pathways of exposure and summarise known effects on anthozoan cnidarian. Finally, we will describe ecological impacts associated with these contaminants and evaluate risk posed to marine coastal communities.

Pesticides

Here, we introduced a short explanation about the mainly environmental chemical and respective effects/ impacts. Such as pesticides, industrial organochlorines, oil hydrocarbon and PAHs, trace metals and metalloids.

The second half of the 20th century has seen a massive increase in population growth and a corresponding expansion of world food production. The acceleration of agricultural output can partly be discribed to the introduction of organochlorine (OC) pesticides such as DDT, which eventually allowed for the shift from classical small-scale farming to industrialised agriculture. The use of pesticides in combination with fertilisers has been growing steadily and has become an essential element of modern agriculture, by preventing the spread of pests and exotic species and by enhancing growth potential, resulting in dramatic increases in crop yields. By the late 1960s environmental drawbacks arising from the use of DDT for agricultural purposes, or as a vector of disease control, became apparent and usage of selected persistent OC pesticides (e.g. DDT, HCB, dieldrin and chlordane) slowly phased out in most developed countries. Other classes of pesticides such as organophosphates, carbamates, organotins and pyrethroids soon filled these roles. Today a wide variety of pesticides used in specialised farming globally, with enormous annual application rates (Fig. 3).

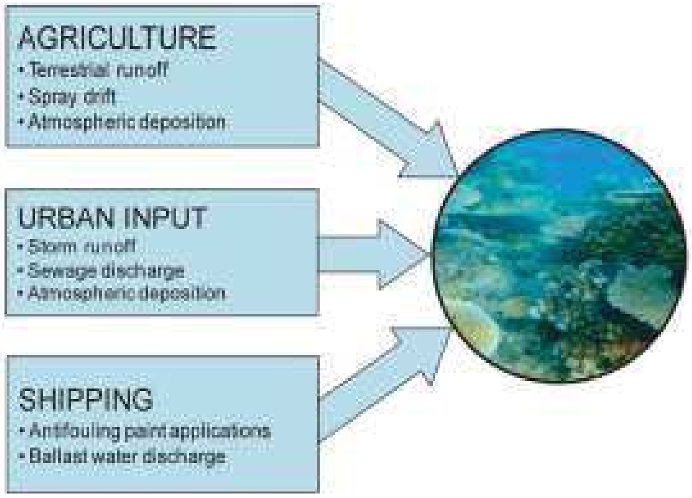

Figure 3. Sources of pesticides reaching coral reef sites.

Modern pesticides generally exhibit shorter half-lives in the environment when compared with their predecessors. Improved usage patterns and progressive application techniques, along side highly specialised modes of action, make unintended side effects to non-target species considerably less of a concern. However, care must observed. The economies of countries in tropical areas often based on agriculture, and depend on intensive use of pesticides to maintain and improve production. As agricultural activities are primarily concentrated in river valleys and coastal plains, it is not surprising that agrochemical residues originating from terrestrial applications are ubiquitous in streams and rivers, eventually draining into estuaries and coastal seas). Multiple studies have shown that the main source of pesticides in nearshore coastal areas is agricultural application, transported via terrestrial runoff (especially during the monsoon season). The effects of pesticide residues are of great concern as many of these compounds and their breakdown products have reported to influence both human and environmental health. Therefore vital to characterise transport and fate of pesticides and their toxicity to non-target organisms to confidently assess risk associated with application, especially in tropical areas where pesticide usage patterns are generally much higher than in temperate zones. Figure 2 provides an overview of sources of biocides reaching reef sites (Van Dam *et al*., 2011).

In addition to terrestrial sources, biocides intentionally released into the marine environment by incorporation into antifouling paint formulations where they function to prevent unwanted growth of a wide range of algae and invertebrates on boats or other marine structures.

Until 2000, most studies on pesticides in tropical marine ecosystems focussed on OC compounds, as these persistent chemicals have dominated the pesticide market for decades in tropical areas. Studies also focused on estuaries, as this is likely to be where most terrestrially derived sediments settle and therefore where most concentrated reservoirs of persistent chemicals will detected.

The limited studies assessing banned persistent organochlorine substances on coral reefs found concentrations to be generally low (Glynn et al., 1973; Glynn et al., 1993) or declining. While organochlorine residues (e.g. endosulfan) been measured in recent times, it is the current generation of pesticides that are of greatest concern to inshore Marine ecosystems.

Recent studies have found contemporary herbicides to be ubiquitous in waters nearby coral. Systemic herbicides are of particular ecological concern to coral reef systems, as these compounds are developed for quick environmental uptake through the root system of plants and are therefore relatively water-soluble. A chemical's solubility will determine whether it mainly exists in the water column or is associated with suspended particulate matter, and therefore more likely to sink to the bottom. In contrast to the latter fraction that precipitates nearshore and becames incorporated in the sediment, chemicals dissolved in the water column can travel greater distances and exert adverse effects far from their application sites. Thus, apart from a greater potential to reach reef sites, herbicide pollution can have severe consequences for ecosystems dependent on primary production (Van Dam *et al*., 2011).

Impact

Little known regarding the effects of OC pesticides on reef building corals. Suspected adverse effects caused by OCs range from carcinogenesis, interruption of neurological function, changes in cell metabolism and gene expression, to endocrine disruption and interference with reproduction. In an early study on organochlorine pollution, McCloskey & Chesher observed photosynthetic depression in a number of scleractinian corals after *in situ* exposure with DDT, dieldrin and a PCB with concentrations in the mg/L range. However, even these extremely high concentrations did not result in alterations of feeding behaviour, polyp expansion, sediment clearing or skeletal crystal formation. Olafson explored bioaccumulative potential of OCs in reef biota and found DDT, chlordane and lindane able to accumulate in coral tissues. In a recent study assessing effects of short-term exposure (up to 96 h) to low insecticide concentrations on different life history stages of the branching coral *Acropora millepora*, endosulfan (OC), chlorpyrifos and profenofos (OPs) were found to affect photosynthetic performance and/or density of zooxanthellae within adult branches at relatively high concentrations (Van Dam *et al*., 2011).

Industrial Organochlorines: PCBs, Dioxins and Furans

Polychlorinated dibenzodioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) are ubiquitous organic contaminants. This group of chemicals is extremely persistent, has a tendency to bioaccumulate in biotic tissues and includes some of the most toxicologically potent compounds known. Although a global treaty aimed at the reduction and possible elimination of PCBs and dioxin-like chemicals has established in 2001, significant concentrations still found in marine environments worldwide. Evidence exists that distribution of PCBs, PCDD/Fs into the aquatic environment is mainly due to atmospheric deposition of volatilised molecules. Despite many studies in temperate and arctic regions and extensive reporting on the concentrations and some effects of PCBs and PCDD/Fs in tropical marine species including mammals, fish and invertebrates, to our knowledge no studies have been performed assessing concentrations in tropical waters or on coral reefs and effects on reef-building corals remain speculative. Dioxin-like substances bind to the aryl hydrocarbon receptor in vertebrates and invertebrates, resulting in interference with a broad range of cellular processes. Concentrations of these compounds in water are presumably too low to cause direct effects on primary producers and environmental impact is mostly associated with bioaccumulation, affecting air-breathing organisms at the top of the food chain. Therefore, likely ecological impacts of dioxin-like pollutants on coral reefs will consist of top-down imbalance of the system (Van Dam *et al*., 2011).

Oil Hydrocarbons and PAHs

Crude oil, refined petroleum and their combustion products all contain aromatic hydrocarbons, including some polycyclic aromatic hydrocarbons (PAHs). These compounds enter the marine environment through anthropogenic processes or have naturally occurring sources. A study by Capone and Bauer suggested that in the late 1980s an estimated average of 6 million metric tons of petroleum products released into our oceans annually. As most petroleum products are hydrophobic in nature, the majority of aromatic hydrocarbons introduced into the marine environment will associate themselves with particulate matter and deposited in the sediment, where these compounds tend to persist. Benthic filter feeders or sessile organisms are at risk through direct contact or ingestion of oil compounds. Straughan argued the biological consequences of oil spills should determined by the nature and interaction of a multitude of factors, including type of oil, dosage, remedial action, prior exposure, presence of other stressors, differences between biota and many physical environmental, climatic and seasonal factors. Generally, a mixed product containing a broad spectrum of hydrocarbons released to the marine environmental where it may affect a variety of biological processes. The potential of oil contamination to coral reefs is high given their often-intense commercial activity and proximity to shipping lanes. Acute exposure of reef ecosystems to oil spills can occur through accidental discharge from ships or because of terrestrial runoff. Production platforms in the vicinity of coral reefs have the potential to contaminate surrounding waters through discharge of production formation water (PFW), a complex mixture that may contain petroleum hydrocarbons, suspended solids, metals, naturally occurring radioactive materials, organic acids and inorganic ions amongst other substances. Likewise, the drilling of oil wells may introduce mud heavily contaminated with petroleum hydrocarbons and metals to the marine system, while refineries and pipelines are additional sources with potential for chronic pollution by oil products. Floating oil be deposited on reef flats or to interfere with reproductive processes in buoyant gametes or larvae. Large oil spills often moderated by application of surface dispersants that dissolve slicks into smaller droplets. However, application of dispersants is likely to increase hydrocarbon concentrations in the water column and thus increase exposure to benthic reef organisms. Once in the water column petroleum hydrocarbons rapidly become associated with organic matter and suspended particles. Volatile components evaporate while non-volatile components deposited into the sediment. This deposited fraction is unlikely to absorb, evaporate, dissolve or be biologically degraded. Oil products may also occur in globulised form disperse through the water column and can settle onto the reef. Aromatic hydrocarbons, either in the form of dispersed oil or as water soluble components, can be absorbed by coral tissues while oil globules can adhere to coral surfaces. The high lipophilicity of aromatic hydrocarbons stimulates rapid passive uptake in the coral tissue, while detoxification can be slow. Residues of aromatic hydrocarbons been reported to remain present in coral tissues months after exposure occurred, yet evidence suggests hydrocarbon deposits in sediments and coral tissues to be substantially reduced after two years at high-energy reef sites (Van Dam *et al*., 2011).

Impact

Adult coral colonies can be kill or injured by direct contact with oil or drilling mud. Filter feeders and benthic organisms that cannot escape the oil or contaminated sediments will typically see bioaccumulation of toxic compounds, genetic mutations and metabolic disorder in their tissues. Corals exposed to hydrocarbons been shown to exhibit loss of zooxanthellae (bleaching), impaired reproduction and tissue damage. Field studies on mud discharges during oil well drilling found decreased coral growth rates in *Montastraea annularis* exposed to the fluids, while several years of exposure found 70 to 90% reduction in coral cover within one hundred meters from the drilling site. These findings supported by laboratory studies predicting decreased growth, metabolic aberrations and nutritional abnormalities. More recently, Raimondi and colleagues (1997) observed tissue mortality in adult cup corals after exposure to drilling muds. Bak (1987) observed decreased coral cover, diversity and local recruitment after chronic exposure to refinery petroleum on a Caribbean reef and argued chronic pollution to have more severe effects than single spills, yet comparable detrimental effects were observed after an acute major oil spill in Panama. Guzmán and co-workers (1994) also observed a strong correlation between injured corals and oil residues in sediment during five years of monitoring coral health after this spill. Aromatic hydrocarbons been shown to decrease photosynthetic performance of dinoflagellate symbionts in some corals (Cook and Knap, 1983; Neff and Anderson, 1981). Jones and Heyward (2003) observed decreased photochemical efficiency and subsequent expulsion of zooxanthellae by host corals exposed to PFW because of photoinhibition in the symbionts (all cited in Van Dam *et al*., 2011).
Marine pollution from PAHs and oil hydrocarbons is associated with localized events as runoff from urban centres, oil exploration and extraction activities and accidental spills. These chemicals are highly hydrophobic and thereforecontamination typically remains relatively confined, although spillage from offshore drilling operations can travel for great distances. Likely effects on coral reefs will consist of overall disturbance of biological homeostasis by exerting effects over multiple trophic levels. The greatest impacts on coral reefs are likely to occur if hydrocarbons come into direct contact with coral spawn during mass reproduction or at low tide on shallow reefs. Interactions with other environmental stressors are improbable, as effects from spills and shipping incidents often cause severe mortality and will overwhelm subtle adverse effects from other factors.

Trace Metals and Metalloids

Metals are a physical component of rocks and soils and enter the environment through natural weathering and erosion processes. Many metals are biologically essential, yet most have the potential to become toxic above certain threshold concentrations. Industrial activities such as mining and smelting as well as agricultural applications (*i.e.* organometallic pesticides and fertilisers) and urban waste have substantially contributed to the release of elevated quantities of trace metals into the environment. Even though recognition of toxic potential and legislation in the past have seen a great reduction in metal output, environmental contamination continues.

Terrestrial runoff and sediment-bound transport through freshwater streams and rivers eventually delivers these contaminants to estuaries and inshore seas. Metals are strongly associated with particulate matter and therefore not usually directly available to aquatic biota. However, particulate metals in sediments can be solubilize by acidic juices in the gut of sediment-feeding organisms, and thus become available for accumulation in biotic matrices through passive uptake across permeable surfaces such as gills or the digestive tract. Biological availability and solubilisation rates of trace metals from particulate matter are dependent upon a variety of environmental variables, including sediment cation exchange capacity and organic content, dissolved oxygen concentrations, pH, salinity, temperature and redox potential amongst other factors. Furthermore, remobilisation and resuspension of sediments may return metals to the water column. High environmental metal concentrations are generally restricted to locations adjacent urban centres, industrialised areas or sites draining areas of intensive agriculture (Batley, 1995). Trace metals and metalloids have a multitude of applications and sources, yet the most abundant metals entering the environment in elevated quantities as a result of agricultural activities are copper (Cu) and zinc (Zn), used as constituents of fertilisers or biocides; arsenic (As), cadmium (Cd) and mercury (Hg) as components of some fungicides. Lead (Pb), nickel (Ni), aluminium (Al), manganese (Mn) and iron (Fe) often enter marine waters as the results of mining activities (as do As and Hg), industrial or urban waste discharges and runoff.

Numerous studies exist on metal contamination and effect on corals (Guzmán and García, 2002; Ramos et al., 2004; Reichelt- Brushett and McOrist, 2003; Victor and Richmond, 2005). In adult corals, metals might be absorbed and occur in various capacities. As early as 1971, Livingstone and Thompson (1971) found trace metals to be incorporate into the aragonite (a carbonate mineral) of coral skeletons. Quantification of these built-in skeletal metals currently used as a biomarker that reflects environmental conditions during the coral's lifetime (Al-Rousan et al., 2007; David, 2003; Fallon et al., 2002). Trace metals can also be found in skeletal cavities (Howard and Brown, 1984), integrated within the organic matrix of coral skeletons (Mitterer, 1978), or absorbed onto exposed surfaces of the skeleton (Brown et al., 1991). Besides skeletal inclusion, several studies have demonstrated trace metals present in coral tissue (Howard and Brown, 1987; Bastidas and García, 1997; Esslemont, 1999). Pathways through which corals absorb metals may vary. Brown and colleagues (1991) found that corals retract their tissue in response to environmental stress, and thus may be more susceptible to direct uptake of metals by exposed skeletal spines. Another responsive action to physical or chemical stress is the excretion of high quantities of mucus with a high affinity to bind metals that may actively reduce metal uptake (Dodge and Szmant-Froelich, 1985). Additionally, it been suggested corals are able to regulate internal metal concentrations through the physiology of their zooxanthellae endosymbionts. Studies with related symbiotic organisms like sea anemones indicated zooxanthellae to be responsible for the majority of metal uptake and accumulation (Harland and Nganro, 1990). In response to elevated metal concentrations, zooxanthellae can enhance calcification rates. Furthermore, zooxanthellae may be involved in the active uptake of trace metals, accumulating higher concentrations of metals than do host coral tissues (Harland and Brown, 1989; Reichelt-Brushett, 2003; Livingston and Thompson, 1971; Marshall, 2002). Subsequent stress-induced expulsion of symbionts by the coral host may act as a regulatory response mechanism in reaction to high metal concentrations (Jones, 1997) (all cited in Van Dam *et al*., 2011).

Impact

Bioavailability, physiological effects and fate of trace metals are highly dependent on the chemical form and oxidation state in which metals exist, as reflected by their toxicity. Thus, it is of clear importance to distinguish between individual metal species present in a particular biological compartment. Once introduced in a biotic matrix, trace metals have the potential to affect nutrient cycling, cell growth and regeneration, as well as reproductive cycles and photosynthetic potential. Elevated levels of copper, zinc and tin in the effluent of a tin smelter in Thailand caused reduced growth and calcification rates in branching corals. In a study considering corals in a Hong Kong estuary exposed to elevated concentrations of metals, pesticides, nutrients, sewage effluents and suspended sediments over a prolonged period of time, it was argued metals were mainly responsible for declines in coral cover, diversity, abundance and growth rates. Laboratory exposure of the massive coral Porites lutea to elevated iron concentrations resulted in bleaching. It noted that corals that been pre-exposed to an iron-enriched environment responded in a less drastic way, suggesting development of some form of iron tolerance. Jones (1997; 2004) found elevated copper concentrations to induce rapid bleaching in the branching corals *Acropora formosa* and *Seriatopora hystrix*, while no inhibition of photosynthetic efficiency of zooxanthellate endosymbionts was observed. The author suggested copperinduced bleaching to occur without affecting the algal photosynthesis but may be relate to effects on the host coral (all cited in Van Dam *et al*., 2011).
Trace metal pollution is often limited to areas adjacent to urban and industrial centres or near river deltas. As metals are relatively immobile in the marine environment, adverse effects are likely to be exerte on a localised scale. Copper and organometallic substances containing tin or mercury are significantly more potent than other trace metals and affect a broad range of variables in a variety of species. Consequences for the system will consist of both bottom-up and topdown effects. Chronic, low-level metal contamination may decrease resilience of marine organisms to other environmental stressors such as elevated temperatures, ocean acidification and other chemical pollutants.

The second chapter treat of natural populations exposed to many contaminants pollution sources comparatively to control previously established. Studies with natural populations (PCBs and aromatic hydrocarbons) it was improve in order to answer if is possible to create a databasis of bioaccumulations levels on organisms from different climatic zones. The specific objectives was evaluate the organisms bioaccumulations patterns to chemical stressors exposition. The hypothesis sea anemones populations could be an early signalizing to environmental changes for chemical stressors, as evidenced by sea anemones bioaccumulations patterns, could be variety to climatic zones provenience. Finally, exist differences on bioaccumulation patterns between congener species, but all species answer to an environmental change before that the effects it il be deleterious to next generations.

Based on the contaminants dispersions models, the present study try to affirm that sea anemones bioaccumulations values present latitudinal variability, according to climatic scenarios different conditions (Fig.4). On the chapter two, we present the mainly article about the chemical stressors studies to natural populations.

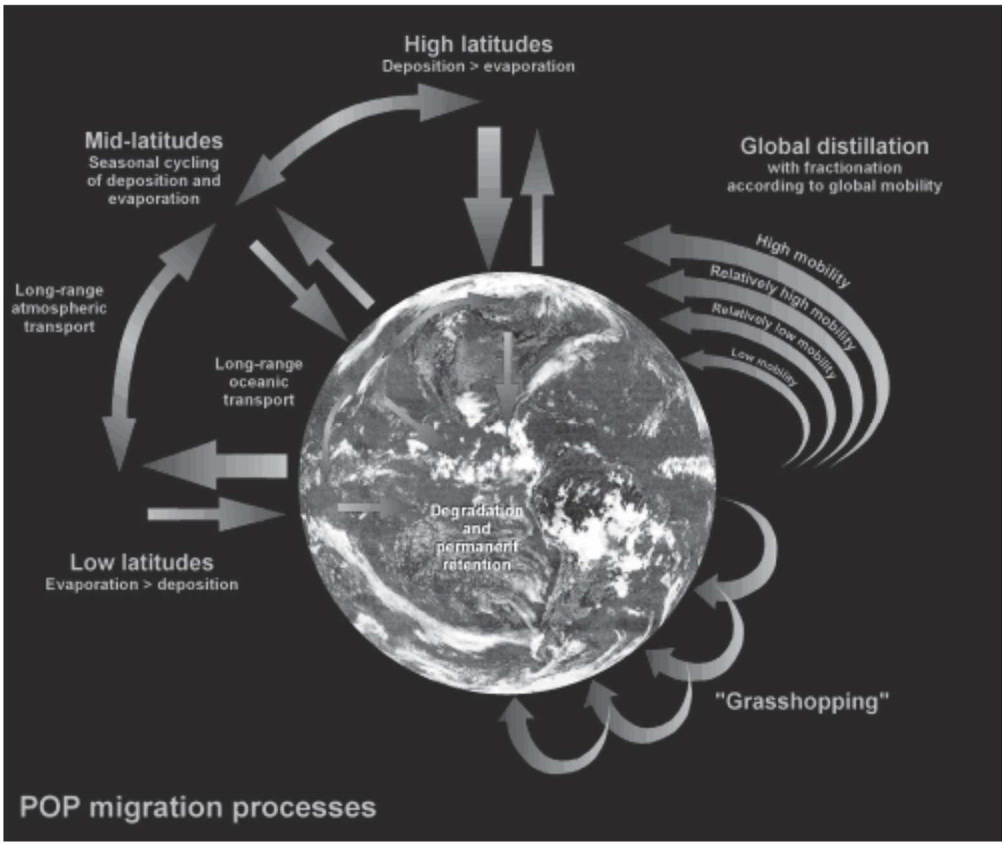

Figure 4. Modify POPs migration processes scheme variations by latitude according to Shiedeck et al (2007).

1.1.6. Effects of Chemical Stressors on Biochemical and enzymatic function on marine organisms

The present study research effects of chemical stressors on sea anemones natural populations from three distinct climatic scenarios. Where, the temperature indirectly was tested on field and directly tested on laboratory assays. It knowed that the temperature fluctuate between the tropical, subtropical and temperate zones. Samples were collected and posterior analysed on laboratory in order to quantify: 1- bioaccumulations levels/ contaminant concentrations; 2- quantification of enzymatic activity and 3- dected histochemical patterns. Inside each climatic scenario, it was identified a control and polluted places characterized according environmental characteristics. The present work combined field and laboratorial studies, in order to answer the proposal hypothesis. The effects of stressors it was tested in two ways: 1- environmental contaminant quantification (Bioaccumulation levels[1]) and effects quantification (Enzymatic activity[2]) on natural populations from three climatic scenarios with laboratorial assays and physical (temperature[3]) effects under controlled conditions.

For the field studies, the chemical quantification and enzymatic activity it was identified and quantified using a pollutants and biomarkers procedures described on chapter 3 (paper 2) and chapter 4 (paper 3), respectively.

The mainly question proposed was that if exist significantly differences on bioaccumulations levels and enzymatic activities differences between sea anemones natural populations from three distinct climatic scenarios.

1- paper 2; 2- paper 3; 3- paper 4.

The factor in commom between the three climatic scenarios is the temperature differences. The present study proposed to investigate if congeners sea anemones species could answer of the same way to chemical and physical stressors, or if them have adaptations to can support this global temperature fluctuations. Exist a such of cellular mechanisms of compensation to temperature and respective enzymatic adaptations. At the cellular level the process of temperature acclimation is the result of complex reorganization of cell metabolism. Such changes depend upon steepest geographical thermal gradient in the world. Populations of the anemone show a graded sequence in differences in allele frequencies at several enzyme loci across this thermal gradient. One example is the an allozyme is selectively favoured in warm water (Blaxter and Southward, 1997).

Enzyme kinetics- temperature changes frequently have substantial effects upon the equilibrium constants of biochemical reactions, particularly those the reversible formation of non covalent (or weak) chemical bonds. It would be expected that natural selection will have produced the altered enzymes in response to changes in environmental temperature so that they may maintain normal function as much as possible. The conservation of kinetic properties of enzymes isolated from different biological system has been demonstrated in a number of studies comparing the temperature responses of homologous protein isolated from organisms adapted to different environments (Blaxter and Southward, 1997). Comparision of closely related species in habitats differing in temperature offers an experimental approach for investigating differences in the kinetics properties of homologous enzymes, although it is always difficult to rule out the possibility of other reasons for observed differences between species. In the absence of relevant research on coral and sea anemones, the present sudy could be use as an example where we compare enzymatic and bioaccumulations parameters in six sea anemones species living in temperate, subtropical and tropical environments.

Homeostasis- An important feature of temperature-protein adaptations is that a protein must exist in a "semi-stable" state if it is to have the ability to undergo the changes in shape that catalysis and regulation require (Hochachka and Somero, 1984). Acclimation to high or low temperatures involves changes in the composition and percentage of unsaturated lipids to maintain the homeoviscous state. At low temperatures, membranes are more likely to enter the gel phase; the incorporation of unsaturated lipids increases the double bond content and lowers the transition temperature between fluid and gel phases. Incorporationg saturated lipids ha the opposite effect, and helps to maintain the homeoviscous state at high temperatures. As expected, the lipids of temperate and cold-water anemones are more unsaturated than those in warm-water forms (Bergmann et al., 1956; Blanquet et al., 1979; Harland et al., 1991). Kellogg and Patton (1983), tested a tropical sea anemone Condylactis gigantean, where they observed that the lipid droplets showed a high degree of saturation of lipid classes extracted from both host and zooxanthellae. Patton et al (1977), also founded the same result for the coral Pocillopora damicormis, detecting the unsaturated lipids. Both results could be indicate that external dietary lipid source whereas the saturated lipids are produced by the zooxanthellae, determining the occurrence of unsaturated lipids in corals

(Blaxter and Southward, 1997). Such a conclusion reflects an inherent problem in all the above studies, namely that the analyses conducted are usually bulk rather than membrane fatty acids.

As well as playing a role in homeoviscous adaptation, saturated fats are very resistant to oxidation compared with unsaturated lipids. As such, they may be benefitial to shallow water tropical organisms living in high solar irradiance where photo- oxidative effects are a hazard (Shick, 1991).

Although there is evidence of lipid differences and membrane properties associated with intraspecific variation in resistance to extreme temperatures in plants (Murata and Yamaya, 1984 in Blaxter and Southward, 1997), nothing is known of the roles that these factors may play in corals and their symbiotic algae. An example is the Gulf Arabian corals species those live in a range of temperature of 24.8 ºC, they must have evolved a remarkable suite of cellular mechanisms to achieve a wide temperature tolerance.

Somero (1978) considered that a complete understanding of temperature adaptation can be achieved only if one can cncompaass the enzymes, the solutions that bathe them and membrane lipids, together with interactions between these components. To this, Hoffman and Parsons (1991) added: “enzymes need to be studied in situations that are extreme if responses to environmental stress are to be understood at the protein level”. It would seem, then, that for a better understanding of the thermal biology of corals, cellular mechanisms of adaptation offer a fertile area of research endeavor.

The present study could be useful in order to “complete” and increment the marine organisms data bases with valuable information to understand the suit effects on species of global temperature variations and environmental changes.

Physiological and biochemical adaptations- During photosynthesis, chlorophyll is excited to the triplet state which can then interact with oxygen to give rise to potentially toxic singlet oxygen. As discussed that, defensive enzymes such superoxide dismutase (SOD), catalase and ascorbate peroxidase in zooxanthellae and SOD and catalase in the host, together with carotenoid pigments in the algae, act in concert to deactivate these toxic compounds (Blaxter and Southward, 1997).

Pollutants are also known to cause oxidative stress resulting from contaminant uncontrolled reactive oxygen species (ROS) production or an imbalanced state between antioxidant defenses and ROS production is seen occurring in living organisms (Fukada et al. 2011). The combined effects of chemical and forced natural factors such as climatic shifts may cause significant ecological risks and adverse health effects in wildlife populations, namely on diversity, fecundity and reproductive competence (Moreira et al. 2004). Organisms have developed an evolutionary capacity to tolerate environmental variability and changes by means of several mechanisms, such as behavioral adaptations, morphological alterations (intra and interspecific variation), regulation of reproduction, and cellular responses (Parmesan, 2006). Aquatic organisms exhibit a variety of changes in enzymatic antioxidant defenses after exposure to pollutants with oxidative potential (Regoli et al., 2002). Prominent among these antioxidant defense system are the superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione-S-transferases (GSTs). In addition, Lipid peroxidation (LPO) levels have been used successfully as a measure of xenobiotic-induced oxidative stress (Lucesoli and Fraga, 1995; Reinheckel et al., 1998).

On the chapter 3, we was to work with reliable tools to chemical assessment effects, the enzymatic biomarkers. The biomarkers definition is they give information to xenobiotic to any biochemical, anatomic and physiological alteration. The Environmental/Ecological Risk Evaluation integrate pharmacological and toxicological interactions. The environmental biomarkers could be integrate on time/space episodes and to provide quickly answer to exposition (short and long time effects). On chapter 4, we was integrate such types of biomarkers: exposition and effects. The first one indicate that a toxic affect the organisms (quantitative), e.g: Gluthatione; another biomarker type was of effect, this indicate that a toxic already produced a damage on organism, e.g; ChEs. Using the enzymatic biomarkers on tests assays, field or laboratory, we can discuss about the earlier warning answer depend of correct chose of ideal biomarkers type to the specific environmental event.

On the Chapter 4, the goals were to determine enzimatic activity on natural populations using congener species from three distintic climatic scenarios. Evaluate the local chemical exposition effects to many pollution sources (Industry effluents, Harbor, Agricultural, Petrochemical and Anthropogenic). The specific objectives was to determine basal levels reactivity on enzymatic biomarkers; develop a multiplebiomarker to assess a battery biomarkers to discriminate different sources of contamination; evaluate the significate spatial differences on organisms enzymatic biomarkers activities; evaluate the significate interspecific differences on organisms enzymatic biomarkers activities; related the enzymatic activity variation to temperature (latitudinal) differences, according to climatic scenarios. Using the hypothesis that the potential to stress answers give to construct of four functional hypothesis: is possible to stablish a scientific biomonitoring programe based on marine systems enzymatic answers using natural populations from three climatic scenarios. Exist interspecific differences on enzymatic in order to specific species adaptations. The answers patterns could be the same intensity on species found on both climatic scenarios. The enzymatic reactions of oxidative stress could be as an early warning to environmental and global changes.

1.1.7. Physical Stressors

Another parameters tested was the physical stressors, they could have a great influence by an antropogenic disturbances and natural phenomena. The present study focused on investigate the temperatures variations effects on behavior of temperate sea anemone *Actinia equina* as a model to extrapolate the results to natural populations (Chapter 5, Paper 4). Atlantic and Mediterranean warming-related diseases outbreaks and species shifts have recently been documented. Evaluated tools of short-term effects on the health or organisms resistance are necessary to assess and understand mechanisms affecting marine biodiversity. Until now, climate warming has been studied at population or community level. Here we offer a better understanding of such phenomena at the organism level, to interpret effects of natural physical stressors, according to behavioral patterns. Elevated temperature and solar radiation, are now recognized as the primary environmental stresses that lead to mass benthonic organisms, mainly cnidarian bleaching. Intertidal organisms are subject to a variety of stresses such as desiccation, water temperature, acidification, increase salinity, nutrient limitation, space competition and predation. The thermal stress is a knower such a mainly environmental factor responsible for climatic and environmental changes. This study takes a behavioral (morphological and anatomic parameters, with physiological implications) to identifying changes soon after exposure to physical stressors in the temperate sea anemone *Actinia equina*. Sea anemones were subjected to variation temperature range over a 96 hours period. Behavior endpoints were divided to be differentially showed as a function of temperature stress. Behavioral patterns analysis placed the differentially ecological functions in a wide range of categories including tentacle flexion, tentacle retraction, column cavitation, peristome depression and oral disc flexion. This suggests that the early stress response to elevated temperature involve essentially all aspects of same chemical reactions, in this case we observed an receptors functioning and the frequency of open-close oral sea anemones, tentacles and columns anatomic alterations to detect earlier the effects of physical stress induction.

Some studies with effects on natural populations and laboratorial exposures test it was made in order to investigate an effect of symbiotic algae (zooxanthallae) to environmental and physical stressors, namely temperature and UVs effects. The present study, focused only on sea anemones responses to environmental stresses.

Richier et al (2006) in an oxidative stress and apoptotic events during thermal stress in the symbiotic sea anemone, *Anemonia viridis, demonstrate that* evidences of oxidative stress followed by induction of caspase-like activity in animal host cells after an elevated temperature stress, suggesting the concomitant action of these components in bleaching.

Gates et al (1992) in a research with temperature stress causes host cell detachment in symbiotic cnidarians, evidenced with scanning electron microscopy of endoderm of the Hawaiian sea anemone *A. pulchellu* after experimental cold shock, revealed profiles that were interpreted as evidence of exocytosis of zooxanthellae.

Dunn et al (2004) in a heat stress induces different forms of cell death in sea anemones and their endosymbiotic algae depending on temperature and duration; founded PCD and necrosis occur simultaneously in both host tissues and zooxanthellae. Subject to environmentally relevant doses of heat stress. Frequency of PCD in the anemone endoderm increased within minutes of treatment.

Lesser (1997) in a work about oxidative stress causes coral bleaching during exposure to elevated temperatures, demonstrated measurements of photosynthesis in the Caribbean coral *Agaricia tenuifolia,* taken during temperature-induced stress and exposure to full solar radiation, a decrease in photosynthetic performance followed by bleaching. Exposure of corals to exogenous antioxidants that scavenge reactive oxygen species during temperature-induced stress improves maximum photosynthetic capacity to rates indistinguishable from corals measured at the ambient temperature of their site of collection. Additionally, these antioxidants prevent the coral from “bleaching” and affect the mechanism of symbiont loss from the coral host. These observations confirm a role for oxidative stress, whether caused by elevated temperatures or exposure to UV radiation, in the bleaching phenomenon.

Clark and Kimeldorf (1971) studied behavioral reactions of the sea anemone, *Anthopleura xanthogrammica*, to ultraviolet and visible radiations. They test for first time, the behavioral parameters to use on the present study and they concluded ecological interpretation for each endpoint, such as tentacle flexion appears to be a response to specific photoreceptors in that the maximum efficiency for stimulation is in the same spectral region as for many forms with discrete photoreceptors. Tentacle retraction is considered to be a response to absorption of energy by proteins and nucleic acids, as evidenced by its maximum efficiency peak at 280 nm. Oral disc, column cavitation, and peristome responses involve regional muscle action, probably induced by deep photoreceptors rather than by nonspecific effects on cell proteins.

Bae and Park (2014), in a recently review, showed that the development and application of BEWS (Biological Early Warning systems) by using various groups of organisms (such as bacterial, algae, cladocerans, bivalve and fish) and the computational methods used to process the behavioral monitoring data. The present study to intend add a new species into this baseline, using a benthonic key-species and a cosmopolitan sea anemone *Actinia equina*.

1.1.8. Effects of Physical stressors on sea anemones Behavior

The Global warming-related diseases outbreaks and species shifts have recently been documented. The present work evaluate tools of short-term effects on the health or organisms resistance. For this, is was necessary to assess and understand mechanisms affecting marine biodiversity. Until now, climate warming has been studied at population or community level. Here we offer a better understanding of such phenomena at the organism level, using anatomic-morphological approaches to interpret effects of natural physical stressors, according to behavioral patterns. Elevated temperature and solar radiation, are now recognized as the primary environmental stresses that lead to mass benthonic organisms, mainly cnidarian bleaching. Intertidal organisms are subject to a variety of stresses such as desiccation, water temperature, acidification, increase salinity, nutrient limitation, space competition and predation. The thermal stress is a knower such a mainly environmental factor responsible for climatic and environmental changes. This study takes a behavioral (morphological and anatomic parameters, with physiological implications) to identifying changes soon after exposure to physical stressors in the temperate sea anemone *Actinia equina*. Sea anemones were subjected to variation temperature range over a 96 hours period. Behavior endpoints were divided to be differentially showed as a function of temperature stress. Behavioral patterns analysis placed the differentially ecological functions in a wide range of categories including tentacle flexion, tentacle retraction, column cavitation, peristome depression and oral disc flexion. This suggests that the early stress response to elevated temperature involve essentially all aspects of same chemical reactions, in this case we observed an receptors functioning and the frequency of open-close oral sea anemones, tentacles and columns anatomic alterations to detect earlier the effects of physical stress induction.

The factor temperature is a most limited and have strong influence on Cnidarian Phyllum representants, namelly in coral reefs. The sea anemones is investigated in order to identify which the effects could affect the physiology and the survivor or the species.

Spatial variation in sea water temperatures at any one time across reefs has been recorded by several authors (Glynn, 1973; Potts and Swart, 1984; Hayashibara et al., 1993 in Blaxter and Southward, 1997) although there are few sutidies providing continuous data which might be of value in interpreting physiological tolerances of corals to temperature fluctuations. Potts and Swart (1984) tested a range of a maximum of three degrees on temperature for two consecutively years and the authors concluded that at least two water masses affect the Heron Island reef: shallow waters and oceanic by prevaling weather conditions. That suggest that the slope site was permanently exposed to oceanic water, revealing that while corals on the crest and the foot of the slope are only 50 m apart, they appear to be surrounded by quite different water masses with very different water regimes. Such effects are likely to be maximal in the summer when waters in the reef lagoon are generally warmer than the shelf waters (Wolanski and Hammer, 1988 in Blaxter and Southward, 1997). So, in some regions characterized by a lagoon influenced places, the effects of temperature culd be tidal, insolation, salinity changes, water lagoon volume, oceanic influences and seasonal effects, as a result corals in lagoon and outer reef also the remarkable tolerance of some individual colonies to a fluctuating temperature regime.

Some authors suggest that exist evidences that the corals may not be able to adapt rapidly to increased temperatures is the observation that repeat annual summer bleaching of the same coral colonies has been observed in the termal plume of a power station in Hawaii (Coles, 1975; Jokiel and Coles, 1990 in Blaxter and Southward, 1997). In other hands, while such results may be suggestive of an inability to adapt to increased temperature, other interpretations are possible, affirming that the “bleaching” effects also potentially varying from year to year.

An example was the Al-Sofyani and Davis (1992) in Saudi Arabia, has shown that the coral *Echinopora gemmacea* demonstrates seasonal acclimatization, without differences on respiration rate. In contrast, *Stylophora pistillata* from the same site appeared to show no compensatory changes in physiological responses to summer conditions, showing the higher photosynthetic rate and a higher metabolic expenditure in the high-light, high-temperature condition of the summer in comparision to the winter. In conclusion, the authors atribute the intraspecific variation on responses, to the fact that different nutritional strategies adopted by two corals, were one is autotrophic, depending of the zooxanthellae activity and the another may be more dependent on particulate food.

Genotypic or evolutionary adaptations to temperature can be demonstrated by comparing the physiology of species that inhabit different thermal environents or latitudinally separated populations of the same species. In all such cases it is critical that phenotypic adaptations or acclimatizations are excluded from comparisions. This is possible if the previous thermal experience of the experimental organisms is carefully controlled. The importance

of this process is largely unexplored, except in a few cases, but it does point to the ultimate necessity to breed and rear organisms under controlled conditions if clear genotypic adaptation to temperature is to be demonstrated (Cossins and Bowler, 1987 in Blaxter and Southward, 1997).
Coles et al., 1976 research a latitudinal comparisons of coral thermal tolerance with field observations and controlled experiment, they reported that tropical corals from Enewetak had an upper lethal and sublethal limit that was about two degrees centigrade higher than that of subtropical Hawaiian corals. But this study concluded that the diferences between each species temperature tolerance it was related to be species-specific rather than latitudinally based.

Marcus and Thorhaug, 1981 investigated species of same genus from Caribe and Hawaii and observed that exist differences with a bleaching threshold about one degree between them. They concluded that time of collection of specimens and their previous thermal history may be important in the final estimation of thermal tolerance. The additional problem of intraspecific variability in bleaching thresholds also presents a difficulty in the analysis of such data sets.

While we might suspect geographic variations in the lethal temperature tolerances between coral species, much more detailed work remains to be carried out to determine whether or not these differences are genotypically based. Clearly, corals are subject to much wider fluctuations in temperature than has previously been recognized and evidence that corals (as a group) live close to their lethal limits is suggestive rather than strong.

Coral bleaching, described primarily in response to increase sea water temperatures, has received widespread attention during the last decade (Glynn, 1993 in Blaxter and Southward, 1997). The response is not unique to reef-building corals; other organisms that contain algal symbionts react similarly on exposure to stressful environmental conditions.
According to Blaxter and Southward, 1997, on time- scale (1-minutes-hour; 2-hour-days; 3-days-weeks and 4-weeks-months) the temperature effects on phenotipic responses observed could be at: 1- molecular level (induction of stress proteins); 2- cellular (altered enzyme activities) or organism (degradation of zooxanthelae and release); 3- Acclimation of photosynthetic (respiration rates); 4- Organism (seasonal acclimatization of photosynthetic/ respiration rates).
On the chapter 5, we discuss about the controlled tests using a sentinel sea anemone specie *Actinia equina*. After carefully bibliographical research, the laboratorial experiment it was design in order to answer the mainly hypothesis tested on the present study: Sea anemones is an early warning to environmental changes. The

temperature it was chosen to be tested under control conditions. The expected results are that the sea anemones answer positively to a variety ranges of temperatures (10ºC - 30ºC), in relation to behavior parameters, survivor and reproduction endpoints. The results of this work it will be integrate an environmental baseline of effects of physical stressors on marine organisms and could be usefull to compare with other studies and promote a very interesting discussion about the Climate chages in Scientific community (Chapter 5).

On the chapter 5, the goals were to determine behavioral parameters combined to physiological alterations on natural populations laboratorial tests using co-generic key specie from temperate climate zone. Evaluate the physical stressors exposition effects to many temperature (10, 15, 20, 25 and 30ºC). The specific objectives were to determine basal levels temperature tolerance morphological and physiological answers; evaluate significative differences on temperature range effects on organisms; related the behavioral and physiological variation to temperature (latitudinal simulations) differences, according to climatic scenarios.

Using the hypothesis about the potential of temperature tolerance stress effects constructed of four functionals hypothesis: Is possible to establish a scientific laboratorial assays to biomonitoring programme based on marine systems chemical and behavioral answers using natural populations from any climatic scenarios. The answers patterns could be the same intensity on species found on both climatic scenarios. The behavioral and physiologic reactions of physical stressors could be as an early warning to environmental and global changes.

To test this hypotheses, the follow questions was made:

- In relation to control temperature experiment (20°C, supposed ideal temperate species, follow OECD guidelines), it was increased or decreased on nutrients, chlorophylls and physico-chemical parameters in relation to temperatures and time variations?
- Survival and reproduction (juveniles polyps' liberation) was increased or decreased in relation to control temperature on temperatures/time variation?
- Condition index was increased or decreased in relation to control temperature on temperatures/time variation?
- Behavioral parameters showed significant differences in relation to temperatures and time variations?

1.2. Objectives and Thesis structure

1.2.1. General objectives

The thesis structural it divided according specific links between a few questions. To determine if natural populations studies and physical stressors simulation studies could be provide enough information about marine and coastal environment state; and if congeneric sea anemones could be used as early warning to environmental studies and climatic changes predictions.

1.3. Specific Objectives

- Select cosmopolitan target species to environmental evaluation;
- Select samples sites based on differents pollution sources to environmental characterization, distributed along three climatic scenarios;
- Based on previous studies, to select chemicals to future exposed laboratorial tests;
- Quantify environmental chemical on natural populations;
- Evaluate chemical effects on natural populations;
- Colect environmental parameters information on each climatic scenarious namelly: physico-chemical parameters, such as temperature, salinity, conductivity, dissolved oxygen, pH; nutrients such as Cholophylls, nitrites, nitrates and phosphates and geochemical cycles such as particulated and dissolved organic carbon and suspended particulated matter.

1.4. General hypothesis

These work to test the follow hypothesis: natural populations sea anemones could be provide a usefull information about climate and global alterations, using environmental databasis and laboratorial validating studies. Could be using as early warning to environmental changes. The chemical assessment could be variety according to climatic scenarios organism's conditions. The idea to use the congeneric species is for stabilish a model studies to environmental assays and provide a reproducible to any places.

The first article that follow talk about environmental characterizations to futures chemical and physical stress responses.

The thesis structure it was divided on: Chapter 1, is a general introduction and talk about these thesis central theme, namelly climate changes, environnmental and climatic changes, physical and chemical stressors, the effects of the physical and chemical stressors on behavior, bioaccumulation levels, enzymatic activities and histological and hystochemical sea anemones body alterations.

The Chapter 2 introduce the state of the art of three climatic scenarios and some environmental alterations. Constructing an environmental data basis for physical chemical parameters from three different climatic scenarios (paper 1).

Chapter 3 introduces the role of sea anemones responses to chemical stressors and environmental changes (paper 2); Chapter 4 focuses on existing early warning/monitoring systems to enzymatic chemical stressors answers on three climatic scenarios, using enzymatic biomarkers of oxidative stress (paper 3); and Chapter 5 presents a global multi-hazard approach to early warning studies, complementing the studies with a physical stressors, under controlled conditions (paper 4).

The Chapter 6 is a final section of the thesis, report the general discussion of all thesis study, and comprehend a general conclusion.

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CHAPTER 2

Behaviour of Nutrients, Carbon and Suspended Particulate Matter from three Coastal Climatic Areas

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Behaviour of Nutrients, Carbon and Suspended Particulate Matter from three Coastal Climatic Areas

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ABSTRACT

A multiple indicators approach was proposed to characterize nutrients and organic matter spatial dynamics (temperature, salinity, dissolved oxygen, dissolved nutrients, SPM, Chl-a, DOC and POC) in marine coastal waters from broadly large coastal geographic areas in three distinct climatic zones, during May to September 2012. In this study the areas were categorized into ecological types according to their habitats and generic characteristics. The hypothesis tested was that the spatial variation of nutrients and organic matter varies according to the climatic scenarios differences. The physical-chemical results, showed a significant spatial variation among climatic scenarios, observing higher increment on chlorophylls, nutrients and organic matter and spatial differences on abiotic factors. The results showed that the three main environment types identified different patterns of water conditions, latitudinal differences and variety stress sources, and the influence of the climatic patterns of the physic-chemical variations, mainly temperature and salinity, on the other factors in each climatic zone. The results suggested that specific characteristics of SPM in each location were highly contributed by sedimentary materials and could be rather consistent under similar weather conditions. On the other hand, the organic matter pool was a highly dynamic carbon pool that represents one carbon reservoir of substantial interest. The mechanisms of organic matter and nutrient relationships, such as a natural disturbance leading to resuspension, need further research to discriminate the impacts of increasing anthropogenic inputs on biogeochemical dynamic. Overall, despite the relatively high environmental and anthropogenic influences in the studied marine coastal areas, effective processing of different sources and forms of nutrients and organic matter occurred and significantly reduces their signatures. Most of these materials were largely transformed and

decreased in amount probably because of flocculation and removal to sediment, microbial degradation and/or dilution by other organic matter sources prior to export to the coastal ocean.

KEY-WORDS: Nutrients; Carbon; Particulate Suspended Matter; Coastal Climatic Areas.

INTRODUCTION

Coastal areas are highly sensitive against climate dependence and each region is affected by the impacts of climate shifts representing different degrees of vulnerability, climatic phenomena that control dependence of the El Niño Southern Oscillation (ENSO) and high tropical North Atlantic (TNA) Sea surface temperatures (SST). Changes in ocean circulation can affect the regional circulation of shelf and coastal seas, leading either to increase export of nutrients plus carbon from the shallow seas into the open ocean or to increased upwelling of nutrients plus carbon onto the shelf and towards coastal areas (Walsh, 1991; Smith and Hollibaugh, 1993; Borges *et al*., 2005). Temperature, salinity and precipitation regimes as well as a wide range of several physical variables due to tidal cycles and freshwater runoff from land put additional pressures and drive changes in coastal ecosystems (Milliman & Farnsworth. 2011). The local atmospheric and hydrodynamics processes interact in complex ways, influencing the physical and chemical attributes of the water column that regulates biological productivity and community structure and thus the food available to higher trophic levels both in the water column and on the sea floor (Biondi *et al*, 2001; Gray *et al*, 2004; d'Orgeville & Peltier, 2007). The physic-chemical parameters such as light, nutrients, temperature, organic matter and other factors affect directly or indirectly the primary production, increase organic matter and biomass, could influence on consumers, feeding and energy consumer on the marine food web (Valiela, 1995). Nutrient fluxes from land to ocean integrate changes in terrestrial ecosystems, in land use, and in other human activities. The extent to which the effects of both nutrients and organic matter will affect coastal areas depends on the nutrient uptake and production as well as on the rates of biotic utilization, photo-oxidation and organic matter sedimentation. Thus, the availability and cycling of nutrients are determined by an interaction of physical, chemical, biological and climatic processes in an ecosystem. This interaction of processes is important as it determines the forms, transformations, and ultimate fate of nutrients in a given system, and thus water quality. These mechanisms that have been most frequently invoked to explain inter annual and long term variability observed in precipitation and alterations in nutrient cycling, allows the flow of nutrient release, with specific pathways for the transfer of particulate and dissolved organic matter patterns over the Atlantic and tropical South America (Richey *et al*., 1983; Nobre *et al*., 2009; Marengo *et al*.. 2008; 2011, 2012). These activities lead to elevated levels of particulate matter and nutrient levels, which promote phytoplankton growth, increased carbon and hence the total particulate organic carbon (POC) and SPM of the affected system (Falkowski *et al*, 2004; Cermeno *et al*., 2008). Thus, these studies emphasized the importance of characterizing the spatial dynamics of the physical and chemical properties of the marine surface water (temperature, salinity, dissolved oxygen, dissolved nutrients, SPM, Chl-a, DOC and POC) in broadly large coastal geographic areas with distinct climatic patterns.

The use of multiple indicators to study the geochemistry of organic matter are justified because of the great diversity of sources of organic matter, which in addition to domestic production, terrestrial/fluvial and anthropogenic contributions promotes significant differences in the reactivity of organic matter on the basis of their origin, spatial and temporal variability influencing the composition of organic matter. The net biogeochemical concentrations of suspended matter within and through tropical, subtropical and temperate scenarios remain scarce. A prerequisite for management of estuarine and coastal water quality and ecosystem sustainability in response to climate change is the understanding of the properties of SPM, particularly in the coastal boundary zone where large gradients of water density and suspended matter control vertical and horizontal SPM/POC fluxes (Gordon *et al*, 1996). These highly dynamic ecosystems, besides being an important geochemical support for the transport of metals and other pollutants (Hansell, 2005) are characterized by strong physical-chemical gradients, high biological activity and intense sedimentation and resuspension of materials. The water quality in these areas is dependent on the properties of cohesive material in suspension (suspended particulate matter, SPM) which mediates primary productivity, biogeochemical cycles, pollutant dispersal, and ecosystem sustainability (Brunskill, 2009, Passow & Carlson, 2012). Apart from its role in the trophic relations of aquatic ecosystems, suspended particulate matter (SPM) plays other important roles, affecting both biological and physic-chemical processes and may serve as a source or sink of carbon and other nutrients (Walsh, 1991, Cermeno *et al*, 2008). SPM determines turbidity which constrains primary production, and deposited SPM generates benthic anoxia, influencing biogeochemical exchanges and benthic productivity (Syvitski & Milliman, 2007, Hansell, 2005). Most estuarine and coastal SPM are in the form of flocs (low density aggregates made of organic matter, inorganic matter, and water) which are the carriers of particulate organic carbon (POC) through the estuarine system (Jago *et al*, 2007). In addition, total organic carbon, composed of dissolved organic carbon and particulate organic carbon, which is not only an important component associated with the water quality of the stream, but also an indicator of organic contamination. Anthropogenic activities can also influence the levels of suspended particulate matter through waste dumping and sewage discharge (Milliman & Farnsworth, 2011). In theory, contaminants are mainly linked to the fine sediment particle and in the high resuspension zones, while, at low tides, the availability is lower.

Healthy and well-functioning coastal wetlands and marine ecosystems are highly important for around 15% of the world's population relying on marine resources as their main or sole source of animal protein and in particularly coastal communities in developing countries. Sustainable management of coastal wetlands and marine ecosystems also offer a wide range of co-benefits, including shoreline protection, nutrient cycling, water quality maintenance, flood control, habitat for birds, other wildlife and harvestable resources such as fish, as well as opportunities for recreation. The accurate assessment of the variability of SPM, POC, DOC, and Chlorophyll along with physical forcing parameters in coastal areas from different climatic regimes may therefore provide valuable insights for improving our knowledge of the biogeochemical cycle prevailing in coastal ecosystems. The efforts to compare data amongst surveys from different climatic areas are important to detect changes in spatial distribution of water quality and to predict the rate of ecosystem changes. In this paper the primary focus is to provide data on biogeochemical concentrations (Carbon, Nitrogen and phosphorus) and the chlorophyll variability, estimating coastal organic carbon concentrations (including POC and DOC) in selected marine coastal waters of three climatic environments, characterized by different pollution sources, properties and dynamics. The hypothesis tested was that the spatial variation of nutrients and organic matter under the influence of different pollution sources could vary according to the climatic scenarios differences.

MATERIAL AND METHODS

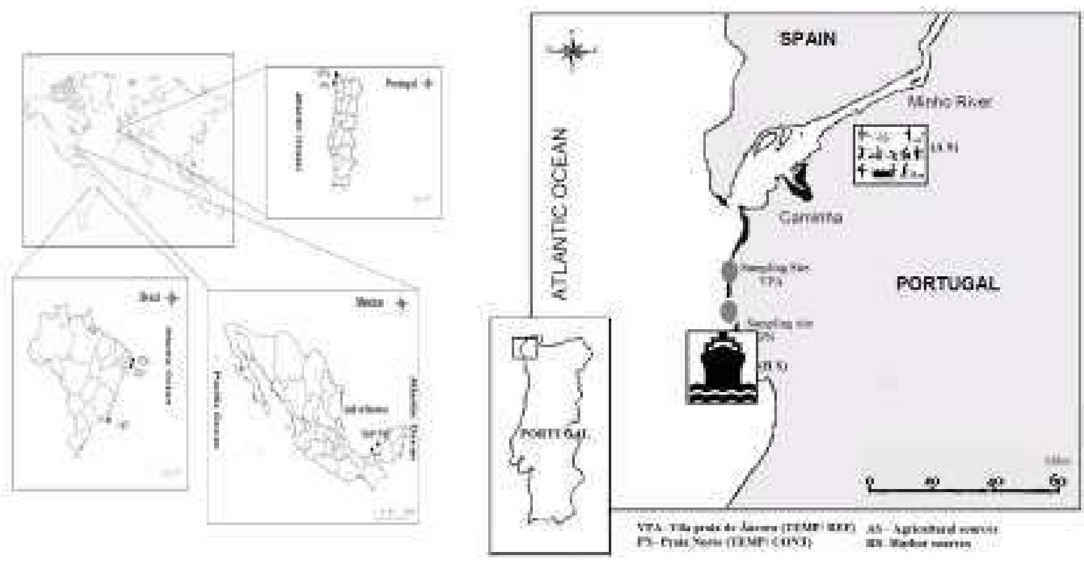
Study Areas

To undertake an integrated monitoring strategy, seven sampling sites were selected in the north, south and western Atlantic coast under the influence of different climatic environments and sources of contamination (Figure 1).

The study locations comprehend reference and contaminated areas under anthropogenic, industrial and harbour influence combining exposed and sheltered habitats.

Sampling sites in tropical environment

In southern tropical environmental (Pernambuco, Brazil) the study was undertaken in Itamaracá (ITA- reference) and was selected as reference station (7º47'S and longitude 34º50'W). It shows a tropical tidal estuarine system (Figure l), located in Pernambuco in north eastern Brazil, 35 km north of Recife, between latitudes 7'34' S and 7'55' S, and longitudes 34"48' W and 34"52' W. It is also a natural and under low anthropogenic influence environmental area (situated in an APA Environment protection area). The other study area in the tropical environment was Olinda (OLI- contaminated) – Northeast coast Brazil the Casa Caiada - Rio Doce beach complex, a 4.5 km- long sandy coastline located at the northern end of Olinda City (Pernambuco, NE Brazil) (Fig. 1), a displayed contaminated area (Pernambuco, Brazil) 7°58'27.21"S; 34°49'54.46"W, described as suffering the influence of anthropogenic and urban and/or industrial contamination pressures (Pio *et al*., 1996). The characteristics of this stressed coastal area were determined, first by those of the surrounding coastal sea and second, by the strong influence of urban activities (Pereira, *et al*., 2003). The abbreviations used for each sample site were: TROP/REF (reference) TROP/CONT (contaminated place).



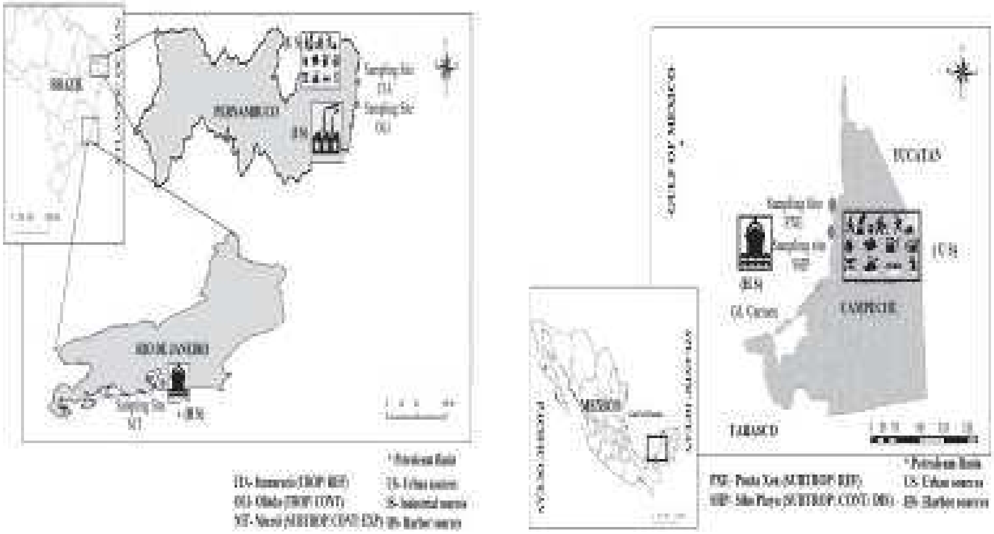


Figure. 1. Location of the sampling sites in the three climatic environments. Northwest Portuguese coast. Vila Praia de Âncora (VPA-reference; Viana do Castelo Praia Norte (PN)-urban and industrial effluents. Brazilian coast, Itamaracá (ITA)- reference, Olinda (OLI)I-urban and Niterói (NIT) industrial effluents. Mexican coast, Punta Xen (PXE)-reference, Siho Playa (SHP) urban and industrial effluents.

Sampling sites in sub –tropical environment

In the southern sub-tropical environment the study area extends throughout two locals in the coastline of México Gulf, eastern part of Campeche (43° 2'56.80"W; 19°20'8.91"N and 90°43'37.92"W; 19°33'24.10"N), Punta Xen (PXE-reference) and southern Siho Playa (SHP-contaminated displayed), respectively. The third sample sites were in the Itaipú bay (22°58'18.10"S and 43° 2'56.80"W) (NIT- contaminated exposed) Niterói (Rio de Janeiro State) in Brazilian coast, (Figure 1, Table 1). The Siho Playa (SHP-contaminated displayed) and Itaipú (NIT-contaminated exposed) point have been described as areas under agricultural, harbor and anthropogenic influences (Castillo 1984; Lavenère-Wanderley 1999. Monterroso, 2005). The abbreviations used for each sample site were: SUBTROP/REF (reference) CONT/ DIS or CONT/ EXP (contaminated place).

Sampling sites in temperate environment

In the temperate environment the study was carried on NW Portuguese coast described as the southern geographical limit for many boreal species and the northern or western limit of subtropical and Mediterranean species (Saldanha, 1974). The two sites selected in a northwest temperate environment were Vila Praia de Âncora, as reference station (VPA- temperate/ reference) (41°49'13.54"N and 8°52'26.05"W), and Praia Norte (PN-temperate/ contaminated) (41°41'41.61"N and 8°51'6.43"W). VPA – Vila Praia de Âncora, is located near small fishery villages and far from big population aggregates and potential sources of contamination (Agricultural, Urban and Harbor). Several studies performed in this coast indicated that this site is relatively undisturbed by anthropogenic pressures (Moreira *et al*., 2005). PN- Praia Norte is located in the vicinity of important industrial facilities, namely an oil refinery and a harbor supporting intensive vessel traffic; thus, they are chronically exposed to petroleum-derived hydrocarbons, including PAHs and heavy metals (Leal *et al*., 1997; Salgado and Serra, 2001; Serra, 1998). The abbreviations used for each sample site were: TEMP/REF (reference) TEMP/CONT (contaminated place).

Table 1. Sampling sites with locations and coordinates information of each climatic scenarios.

Sampling Sites	Location	Coordinates	Climatic Environment
Vila Praia de Âncora (VPA)	Northwest, Portugal	41°49'13.54"N, 8°52'26.05"W	Temperate
Viana do Castelo Praia Norte (PN)	Northwest, Portugal	41°41'41.61"N,8°51'6.43 "W	Temperate
Itamaracá (ITA)	Recife, Brazil	7º47'S, 34º50'W	Tropical
Olinda (OLI)	Recife, Brazil	7°58'27.21"S; 34°49'54.46"W	Tropical
Punta Xen (PXE)	Campeche, Mexico	43° 2'56.80"W; 19°20'8.91"N	Sub-Tropical
Siho Playa (SHP)	Campeche, Mexico	90°43'37.92"W; 19°33'24.10"N	Sub-Tropical
Itaipu (NIT)	Rio de Janeiro, Brazil	22°58'18.10"S, 43° 2'56.80"W	Su-Tropical

Water sampling

The water sampling was carried out between May to September 2012, in the inter-tidal zone (until the 1 m of depth) (Fig. 1). Five liter samples were collected for each sampling site, for all water analysis. The different pollution sources for each location have a relative influence on each sample site. Each one of all the samples sites were situated under "influence zone" (in a Km scale) of the agricultural, harbor, industrial and anthropogenic sources.

Abiotic factors

The following abiotic parameters were measured (three readings for each point) in situ at all the sampling sites, in the rainy season of 2012: salinity (P.S.U) and conductivity (µS/cm) (Wissenschaftlich Technische Werkstätten

– LF 330 meter, Brüssel, Belgium), dissolved oxygen (% saturation or mg/L) (Wissenschaftlich Technische Werkstätten Cell Ox 325) and pH (Wissenschaftlich Technische Werkstätten 537 meter).

Water analysis- Chlorophyll, nutrients, POC, DOC and SPM

Biogeochemical profiles in the bottom water - Distribution of parameters, first improved was Chlorophyll (a, b and c), due to ease in material degradation (photosensitive). The 500 mL of material for each sample sites were previous filtered with a cellulose acetate filter following the Jeffrey and Humphrey`s tri-chromatic Equations (1975). For the nutrient water analysis (nitrites, nitrates and phosphates) three samples for each sample sites were used following the Diazotization Method, Cadmium Reduction and Ascorbic Acid Method (Eaton *et al*., 2005) using powder pillows, respectively. The particulate organic carbon was analyzed using the Treguer & Le Corre (1975) methods and dissolved carbon using TOC- VCPH with catalytic method, read in triplicate samples for each point. The suspended particulate method was SPM- Beta-ray absorption method on previous filtered with GF/F 0.7 um and GF/C 1.2 um filters, in triplicate for each point.

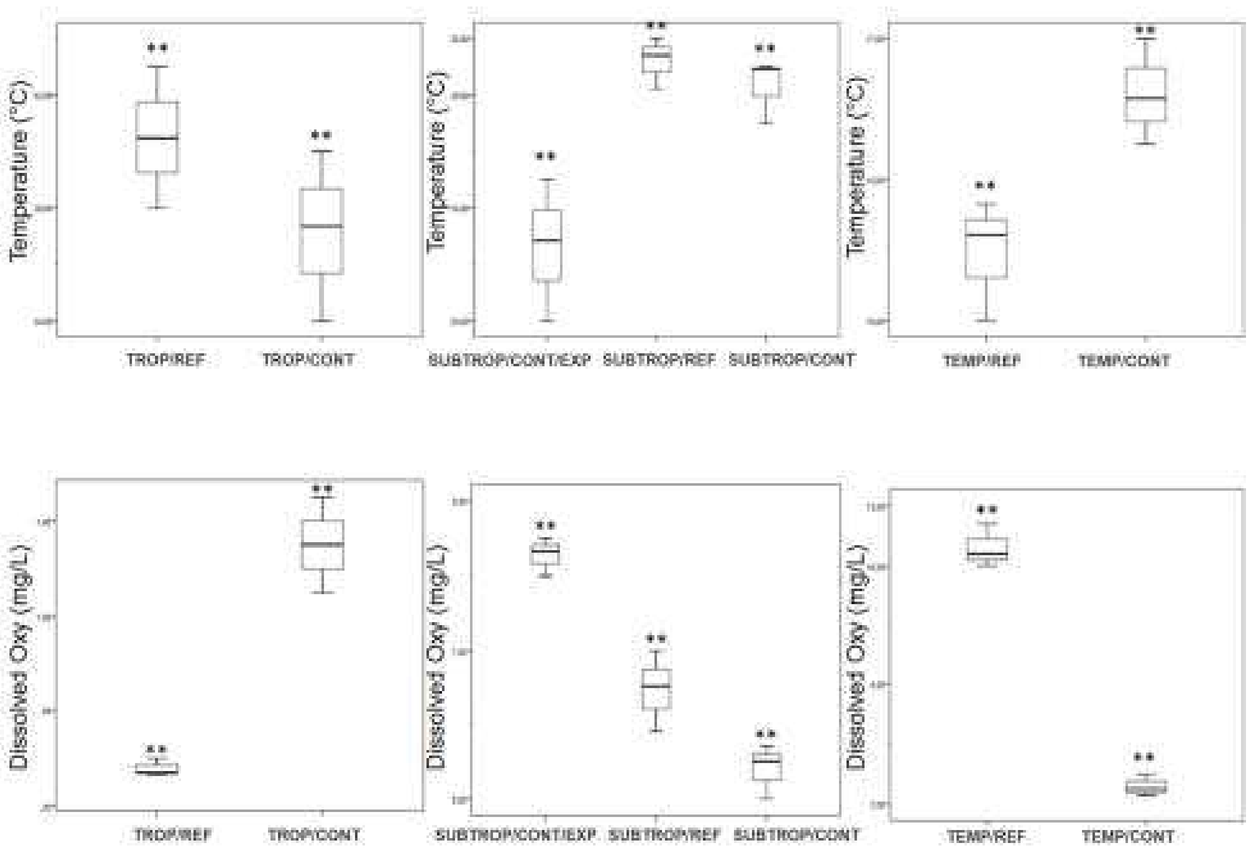
Data analysis

All the measurements consist of three replicated samples for each parameter. The data treatment includes the estimation of maximum and minimum, average and standard deviation. Data for physicochemical parameters of water samples were presented as mean values and analysed using descriptive analysis. Pearson correlation (r) analyses were performed on the individual water quality parameters to identify relationships within each parameter. Different parameters were compared using one-way analysis of variance (ANOVA). Tukey's test was applied if significant differences among different climate zones were detected by ANOVA (Zar, 1996).The normality of data were tested (Kolmogorov Smirnov normality test) and the homogeneity of variance was verified (Barlett's test). For environmental data, the MDS was based on Euclidian distances between samples, after normalization of environmental data. The spatial and species biomarkers responses variations were analysed by Principal Component Analysis (PCO) and compared using a Permutational Multivariate Analysis of Variance (PERMANOVA).The multidimensional scaling (MDS) ordination method was used to visualize the spatial responses. The environmental data were also analysed by redundancy analysis (RDA), using environmental parameters and water parameters as environmental descriptors. Multivariate PERMANOVA, PCO and MDS tests were performed using PRIMER with PERMANOVA+ software (PRIMER v6 and PERMANOVA+ v1, PRIMER-

E Ltd.) The RDA analysis was performed using the software CANOCO 4.5 for Windows (Biometris, The Netherlands).

RESULTS

The results of this study were developed in three different climatic regions, emphasizing the important contribution to the climate knowledge and temperature increase/decrease interferences about dissolved oxygen, dissolved organic carbon, total dissolved carbon, pH, nutrients and suspended particulate material on "natural" dynamics. Water temperature varied from 24.0 ºC ± 6.5, with the highest values registered in the tropical region Itamaracá (ITA-reference) and the lowest values in the temperate region, Vila Praia de Âncora (VPA– reference), 30.47 and 14.20, respectively (Table 2). The highest salinity values were found in the sub-tropical region, Siho Playa (SHP-contaminated displayed), 38.20 psu, and the lowest in the temperate region, Vila Praia de Âncora (VPA–reference), 25 psu (Figure 2) (Table 2).



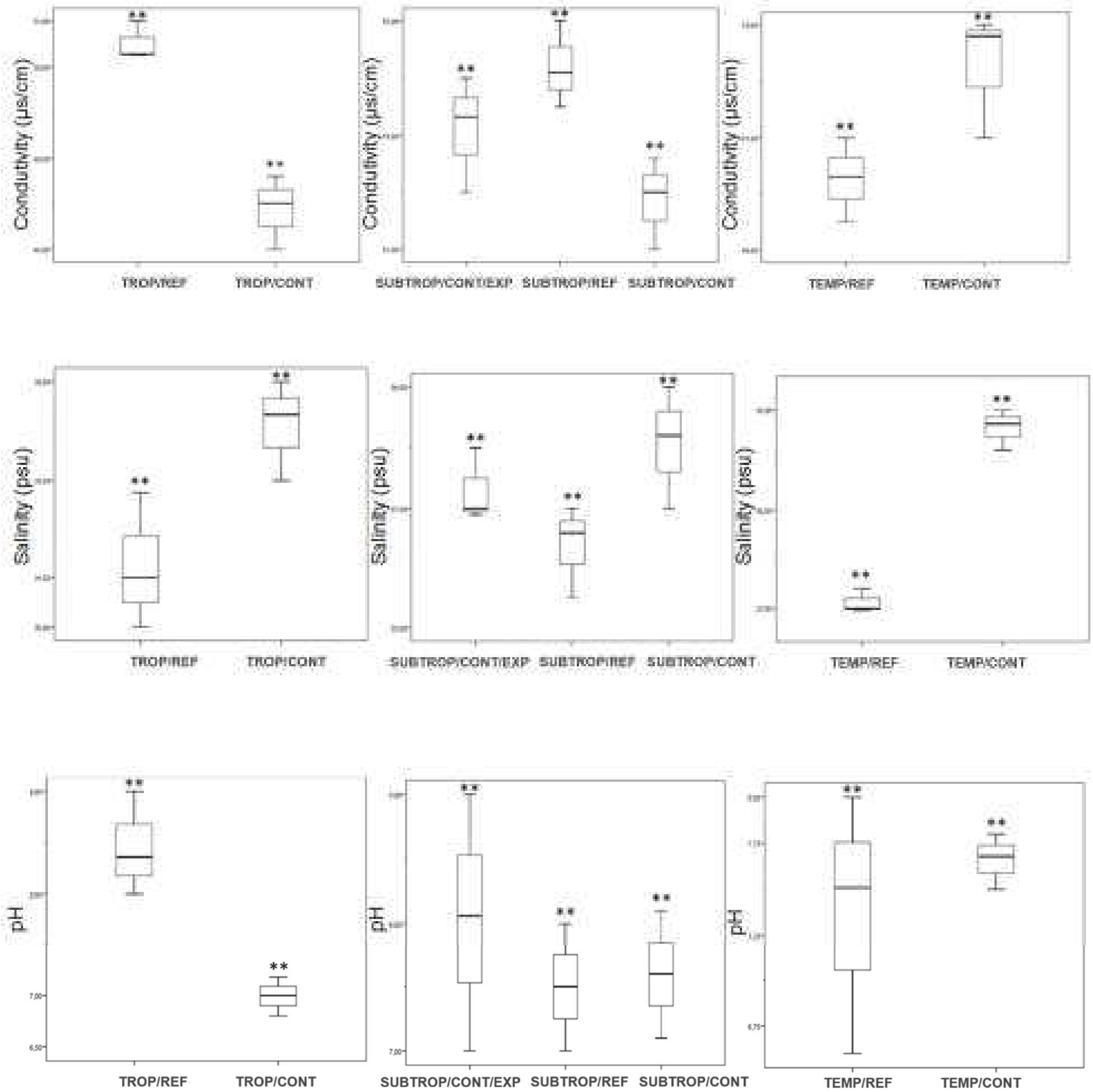


Figure 2. Abiotic factor's boxplot representations for each sampling station on three climatic scenarios.

The highest values for conductivity were observed in sub-tropical region, Punta Xen (PXE-reference) and the lowest values were registered in the tropical region, Olinda (OLI-contaminated), with average values of 51.57 ± 2.27 and 5.42 ± 4.13, respectively (Figure 2) (Table 2). The higher and lower values were registered in the tropical region, Itamaracá (ITA- reference) and Olinda (OLI- contaminated), 8.36 and 7.18, respectively (Table 2).

Table 2. Abiotic factors spatial variation from each climatic scenarios.

Scenarios	Station	Min/Max	Temperature(°C)	Dissolved Oxygen (mg/L)	Condutivity (µS/cm)	Salinity (p.s.u)	pH
Tropical	IT	Min	28.00	0.33	50.26	30.00	8.00
		Max	33.00	0.40	51.00	32.76	9.00

	OL	Min	24.00	1.10	46.00	33.00	6.80
		Max	30.00	1.50	47.60	35.00	7.10
Subtropical	NIT	Min	20.00	8.00	52.00	36.90	7.00
		Max	25.00	8.50	54.00	38.00	9.00
	PX	Min	28.20	5.90	53.50	35.50	7.00
		Max	30.00	7.00	55.00	37.00	8.00
	SHP	Min	27.00	5.00	51.00	37.00	7.10
		Max	29.00	5.70	52.60	39.00	8.10
Temperate	VPA	Min	13.00	1.00	49.50	24.90	6.60
		Max	14.65	11.40	51.00	26.00	8.00
	PN	Min	15.50	2.27	51.00	33.00	7.50
		Max	17.00	3.00	53.00	35.00	7.80

The obtained results also showed that the Temperature presents a significant correlation with Dissolved Oxygen (negative in temperate areas and positive in tropical area, $p<0.05$) (Table 3) and SPM (temperate, tropical and sub-tropical areas, $0.05<p<0.01$) (Table 3), with Salinity (positive in temperate area and negative in tropical area, $p<0.05$); pH, nitrites, nitrates, phosphates and chlorophyll *a* (sub-tropical area, $0.05<p<0.01$) (Table 3). Salinity presented a significant correlation with SPM. In the tropical area, a positive relation was observed (0.878, $p<0.05$) The highest and lowest values were observed in (VPA, reference) and (ITA- reference), respectively. The Dissolved Oxygen presented a significant correlation with temperature, salinity, pH (temperate and tropical areas, $0.05<p<0.01$) (Table 3), and also with nitrites, nitrates, DOC and SPM (Tropical area, $0.05< p<0.01$) (Table 3) and a negative correlation with chlorophyll *a* (sub-tropical area, $p<0.01$). significantly correlated with temperature, nitrites, nitrates and phosphates (temperate and tropical areas, $p<0.05$) (Table 3). Nevertheless, the pH values presented significant correlations with Dissolved Oxygen and Conductivity (temperate and tropical areas, $p<0.01$) (Table 3), and also with Chlorophyll a, Nitrites, Nitrates, DOC and SPM (tropical area, $0.05<p<0.01$) (Table 3) and Chlorophyll *a*, Nitrites, Nitrates and Phosphates (sub-tropical area $0.05<p<0.01$) (Table 3). Values were significantly correlated with salinity in all the coastal areas ($p<0.01$, Table 3) and also with nitrites, DOC and SPM (tropical area, $p<0.05$) (Table 3).

Chlorophyll *a* values presented significant correlation with POC (temperate area, $p<0.01$), pH, nitrites and nitrates (tropical area, $p<0.05$) and also with Temperature, Dissolved Oxygen, pH, nitrites and nitrates (sub-tropical area, $p<0.05$) (Table 3). The Chlorophyll *a,* showed average values of 2.91 mg $m^{-3} \pm 0.36$ in the temperate coastal area, 2.97 mg $m^{-3} \pm 0.36$, in tropical coastal area and 11.69 mg $m^{-3} \pm 0.39$ in the sub-tropical coastal area (Figure 3) (Table 2). The chlorophyll *a*, present the highest values in the sub-tropical region, Punta Xen (PXE-reference), 11,77 mg m^{-3} and Siho Playa (SHP-contaminated displayed), 11.66 mg m^{-3}, and the lowest in the tropical sampling sites, Olinda (OLI- contaminated), 0.31 mg m^{-3}, and Itamaracá (ITA- reference), 0.49 mg m^{-3} (Table 2).

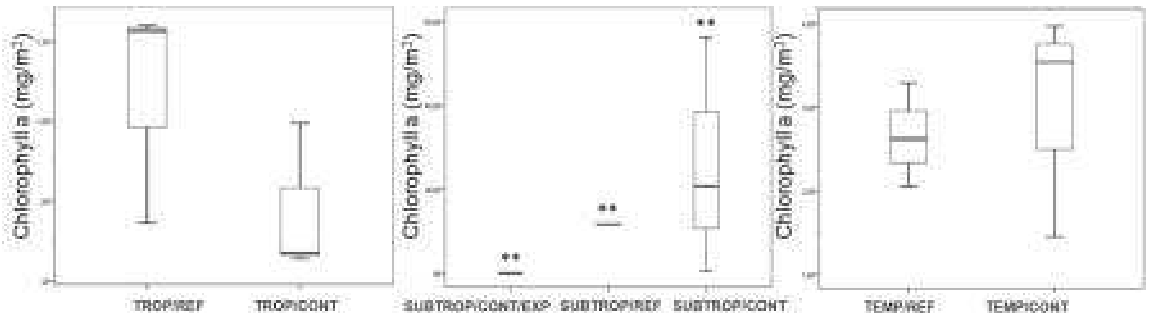


Figure 3. Chlorophyll a boxplot representation for each sampling sites on three climatic scenarios.

The Nitrites value present significant correlation with Salinity, Nitrates (temperate area, $p<0.01$) (Table 3), Salinity, pH, Chlorophyll *a*, Dissolved Oxygen, Conductivity, DOC and SPM (tropical area, $0.05<p<0.01$) (Table 3) and with Temperature, Dissolved Oxygen, pH, Chlorophyll *a*, Phosphates and Nitrates (sub-tropical area, $0.05<p<0.01$) (Table 3). The Nitrate values present significant correlation with Salinity, Nitrites and Phosphates (temperate area, $p<0.05$) (Table 3), Dissolved Oxygen, pH, Chlorophyll *a* (tropical area, $p<0.05$) (Table 3) and also with Temperature, Dissolved Oxygen and pH (sub-tropical area, $p<0.05$). The Phosphate values presented a significant correlation with Salinity and Nitrates (temperate area, $p<0.05$) (Table 3), with Salinity (tropical area, $p<0.05$) (Table 3), and also with temperature, pH and nitrites (sub-tropical area, $p<0.05$) (Table 3).

Table 3. Correlations between all physic-chemical parameters, Chlorophyll, Nutrients, particulate and dissolved oxygenated carbon and suspended particulate matter on each climatic scenario. Significance * ($P<0.05$); ** ($p<0.01$).

Scenarios	**Factors**		**Correlations**	
Temperate	Temperature	Dissolved Oxygen	-0.943	**
		Salinity	0.771	*
		SPM	-0.841	*
	Dissolved Oxygen	pH	-0.759	n.s.
	Condutivity	Salinity	0.771	*
		pH	0.941	**
	Salinity	Nitrites	0.845	*

		Nitrates	0.736	*
		Phosphates	-0.88	*
		SPM	-0.841	*
	Chlorophyll a	POC	-0.771	*
	Nitrites	Nitrates	0.87	*
	Phosphates	Nitrates	-0.813	*
	DOC	POC	-0.771	*
Subtropical	Temperature	Dissolved Oxygen	-0.981	**
		pH	-0.929	**
		Nitrites	-0.985	**
		Phosphates	0.709	*
		Nitrates	0.881	**
		Chlorophyll *a*	0.992	**
	Dissolved Oxygen	pH	0.859	**
		Nitrites	0.938	**
		Nitrates	-0.876	**
		Chlorophyll *a*	-0.992	**
	Condutivity	Salinity	-0.95	**
	pH	Chlorophyll *a*	-0.997	**
		Nitrites	0.975	**
		Phosphates	-0.692	*
		Nitrates	-0.81	**
	Cholophylla a	Nitrites	-0.963	**
		Nitrates	0.906	**
	Nitrates	Phosphates	-0.749	*
		Nitrates	-0.844	**
Tropical	Temperature	Condutivity	0.829	*
		Salinity	-0.771	*
		SPM	-0.878	**
		DOC	0.771	*
	Dissolved Oxygen	Salinity	0.771	*
		pH	-0.841	**
		Nitrites	0.883	**
		Nitrates	0.846	**
		DOC	-0.771	**
		SPM	0.878	**
	Condutivity	Salinity	-0.943	**
		Nitrites	-0.794	*
		DOC	0.771	*
		SPM	-0.878	**
	Salinity	Nitrites	0.833	*
		Phosphates	0.754	*

	SPM	0.878	**
pH	Chlorophyll *a*	0.759	**
	Nitrites	-0.884	**
	Nitrates	-0.889	**
	DOC	0.88	*
	SPM	-0.933	**
	Nitrites	-0.765	*
Chlorophyll *a*	Nitrates*	-0.778	*
Nitrites	DOC*	-0.794	*
	SPM**	0.905	**
Nitrates	DOC*	-0.778	*
POC	SPM*	-0.878	*
SPM	DOC*	-0.878	*

The obtained results for conductivity showed significant variations between and within each coastal area ($p<0.01$, Table 4), and significantly different among the three coastal areas ($F=0.001$, Tukey Pos-hoc test, $P<0.05$, Table 5). The Dissolved Oxygen values showed significant variations between and within each coastal area ($p<0.01$, Table 4), whereas the values from sub-Tropical and Temperate areas were significantly different from ones of the Tropical areas ($F=0.001$, respectively, Tukey Pos-hoc test, $P<0.05$, Table 5). Due to the well-known buffering capacity of seawater concerning pH variations, the pH showed no important spatial variations between the three climatic regions (average = 7.63 ±2.9, $p>0.05$, Tables 2, 3 and 4). Water temperature and salinity results showed that there's significant variations between and within each coastal area ($P<0.01$, Table 4), whereas the values from Tropical and sub-Tropical areas were significantly different from the values of Temperate areas ($F=3.20$, and $F=0.001$, respectively, Tukey Pos-hoc test, $P<0.05$, Table 5).

The most relevant results of chlorophylls type *a*, were shown in Figure 3. Results showed significant variations of chlorophyll *a* values between the coastal areas ($p<0.01$, Table 4) and significantly different among the three coastal areas ($F=0.001$, Tukey Pos-hoc test, $P<0.05$, Table 5). It was observed that there's important variations in the nutrient concentrations, with significant variations in nitrates and phosphate values between each coastal area ($p<0.01$, Table 4).

Table 4- Results from One Way-ANOVA statistical analysis for each parameter measured in water.

	Sum of Squares	df	Mean Square	F	Sig.

	Between Groups	673,166	2	336,583	69,205	,000
Temperature	Within Groups	87,544	18	4,864		
	Total	760,710	20			
	Between Groups	146,266	2	73,133	11,784	,001
Dissolved Oxygen	Within Groups	111,708	18	6,206		
	Total	257,974	20			
	Between Groups	69,964	2	34,982	26,857	,000
Conductivity	Within Groups	23,446	18	1,303		
	Total	93,410	20			
	Between Groups	210,802	2	105,401	13,779	,000
Salinity	Within Groups	137,693	18	7,650		
	Total	348,494	20			
	Between Groups	,099	2	,050	,329	,724
pH	Within Groups	2,708	18	,150		
	Total	2,807	20			
	Between Groups	266,087	2	133,044	6,345	,008
Chlo a	Within Groups	377,423	18	20,968		
	Total	643,510	20			
	Between Groups	,003	2	,001	3,619	,048
Nitrites	Within Groups	,007	18	,000		
	Total	,010	20			
	Between Groups	16,287	2	8,143	34,748	,000
Phosphates	Within Groups	4,218	18	,234		
	Total	20,505	20			
	Between Groups	236,784	2	118,392	5,398	,015
Nitrates	Within Groups	394,766	18	21,931		
	Total	631,550	20			

Differences of nutrients values within Sub-Tropical and Temperate were significantly different from for values from Tropical areas (Tukey Pos-hoc test, $P<0.05$, Table 5). The obtained results for POC showed significant variations between and within each coastal area ($p<0.01$, Table 4), and values within the Tropical and sub-Tropical areas were significantly different from the Temperate areas ($F=0.019$, Tukey Pos-hoc test, $P<0.05$, Table 5).

Table 5- Results from One Way-ANOVA statistical analysis for each parameter measured in water. The Tukey

Parameter	Factor	Statistics	Tukey Comparisons		
Nitrites	Climate area	$F_{(2,18)}$= 0.009 (p<0.05)	Tropical	Subtropical	Temperate
Phosphates	Climate area	$F_{(2,18)}$= 0.001 (p<0.05)	Tropical	Subtropical	Temperate
Nitrates	Climate area	$F_{(2,18)}$= 0.004 (p<0.05)	Tropical	Subtropical	Temperate
Temperature	Climate area	$F_{(2,18)}$= 3.20 (p<0.05)	Tropical	Subtropical	Temperate
Dissolv. Oxygen	Climate area	$F_{(2,18)}$= 0.001 (p<0.05)	Tropical	Subtropical	Temperate
Conductivity	Climate area	$F_{(2,18)}$= 0.001 (p<0.05)	Tropical	Subtropical	Temperate
Salinity	Climate area	$F_{(2,18)}$= 0.001 (p<0.05)	Tropical	Subtropical	Temperate
pH	Climate area	$F_{(2,18)}$= 0.759 (p<0.05)	Tropical	Subtropical	Temperate
Chlorohyll *a*	Climate area	$F_{(2,18)}$= 0.001 (p<0.05)	Tropical	Subtropical	Temperate
POC	Climate area	$F_{(2,18)}$= 0.019 (p<0.05)	Tropical	Subtropical	Temperate
DOC	Climate area	$F_{(2,18)}$= 0.035 (p<0.05)	Tropical	Subtropical	Temperate
SPM	Climate area	$F_{(2,18)}$= 0.000 (p<0.05)	Tropical	Subtropical	Temperate

comparisons results indicate the post hoc comparisons between climate areas. Statistically not different areas are united by an underscore (p<0.05).

Average values of nutrients were 5.61 ± 5.48 uM to nitrates; 0.02 ± 0.02 uM to nitrites and 0.87 ± 0.99 uM to phosphates (Figure 4) (Table 2). Nitrates (NO_3) concentrations registered the highest values in the sub-tropical region, SHP (Siho Playa, SHP-contaminated displayed), 13.33 uM, and the lowest in the tropical and sub-tropical regions, IT (ITA- reference) and NIT (NIT- contaminated exposed), with null values. Phosphates (PO_4) presented maximum values in the tropical region (Figure 4) (Table 2), Olinda, (OLI-contaminated), 2.75 uM and Itamaracá (ITA- reference), 2.74 uM. The other sampling sites presented lower values varying inside the average of 0.87 ± 0.99 uM. Nitrites (NO_2) values outside average were found in the sub-tropical region, Niterói (NIT- contaminated exposed), 0.07 uM (Figure 4) (Table 2).

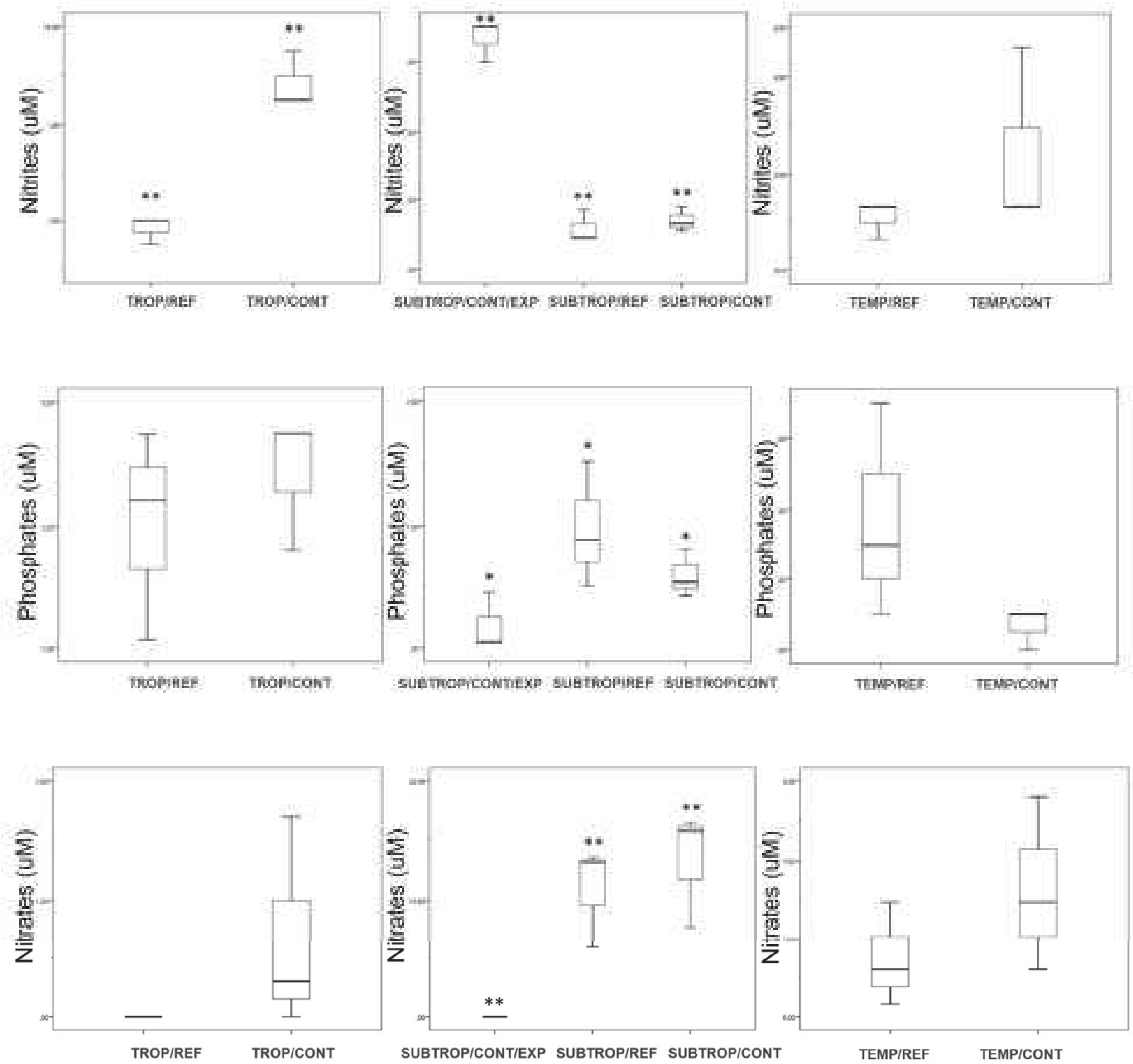


Figure 4. Nutrients boxplot representation for each sampling sites on three climatic scenarios.

The POC values present a significant negative correlation with Chlorophyll *a* and DOC (temperate area, $p<0.05$) (Table 3) and also with SPM (tropical area, $p<0.05$) (Tabel 3) with regard to the particulate carbon (POC) the higher values were found on SHP (Siho Playa, contaminated displayed), 11.73 mg/L ± 2.17, and lower values in temperate sampling stations, Vila Praia de Âncora (VPA-reference, Praia Norte (PN-contaminated), (1.41 mg/L ± 0.26 and 1.40 mg/L ± 0.38, respectively (Figure 5). The obtained results for DOC showed significant variations between and within each coastal area ($p<0.01$, Table 4), and values within the Tropical and sub-Tropical areas were significantly different from the Temperate areas ($F=0.035$, Tukey Pos-hoc test, $P<0.05$, Table 5). DOC values presented significant correlation with POC (temperate area, $p<0.05$) (Table 3), and also with Dissolved Oxygen, pH, Nitrites, Nitrates and SPM (tropical area, $p<0.05$) (Table 3). The dissolved carbon (DOC) higher values were also registered on SHP (Siho Playa, contaminated displayed), 11.63 mg/L and the lowest values in temperate sampling stations, Vila Praia de Âncora (VPA-reference, Praia Norte (PN-contaminated) (Figure 5) (Table 2).

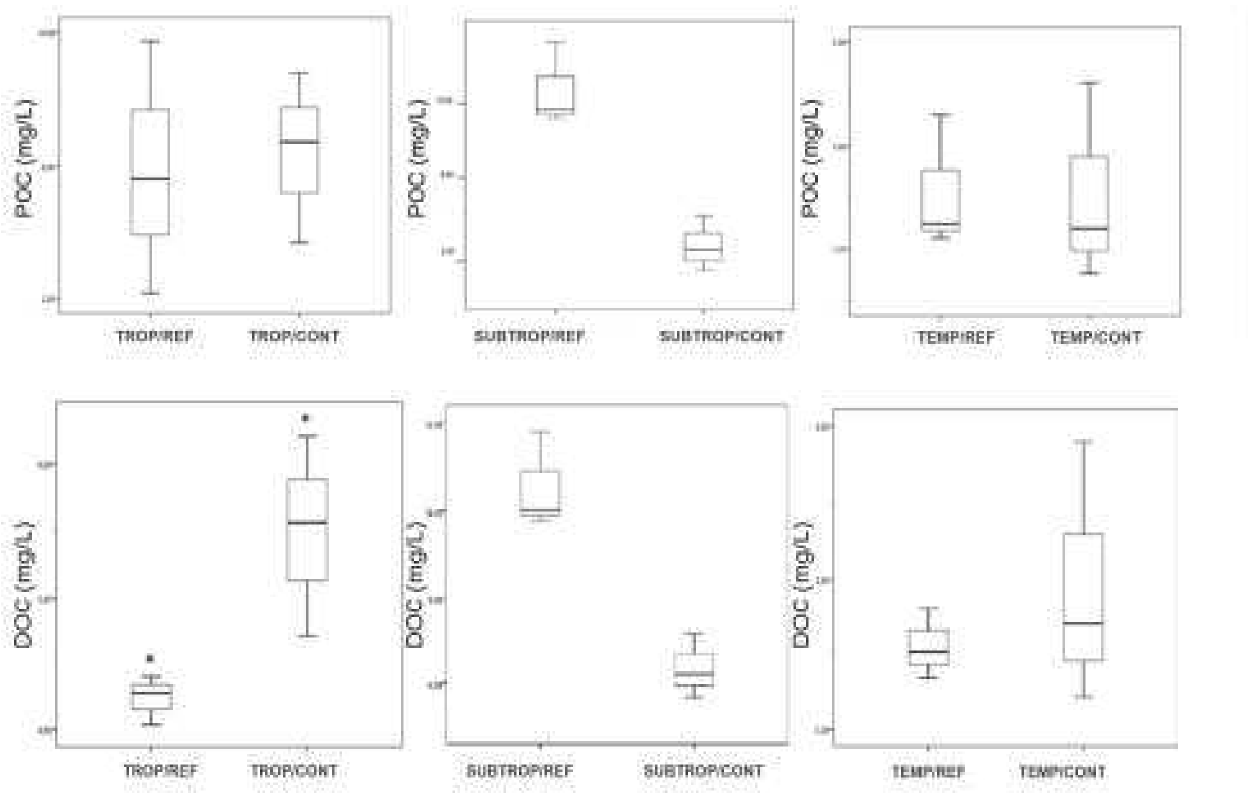


Figure 5. POC and DOC boxplot representation for each sampling sites on three climatic scenarios.

The results for SPM showed significant variations between and within each coastal area ($p<0.01$, Table 4), and significantly different among the three coastal areas ($F=0.001$, Tukey Pos-hoc test, $P<0.05$, Table 5). The SPM values presented significant correlation with Temperature and Salinity (temperate area, $p<0.05$), and also with Temperature, Salinity, pH, Dissolved Oxygen, Conductivity, Nitrites, POC and DOC (tropical area, $p<0.05$) (Table 3). The highest values of suspended particulate matter (SPM) were registered in the control sampling stations, for the temperate, tropical and subtropical areas, Vila Praia de Âncora (VPA-reference) with 23.0 mg/L, Itamaracá (ITA- reference), with 6.06 mg/L and Punta Xen (PXE-reference) with 8.61 mg/L (Figure 6) (Table 2).

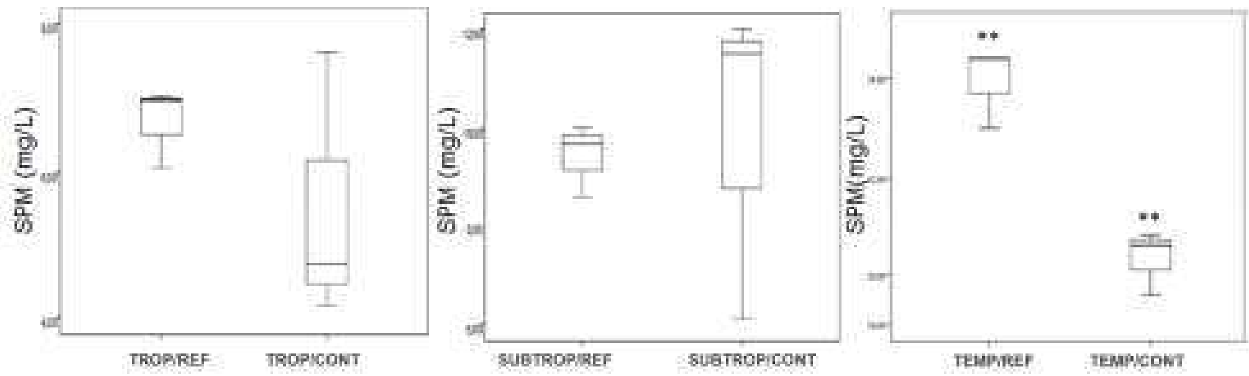


Figure 6. SPMs boxsplot representation for each sampling sites on three climatic scenarios.

MDS (Multiple Dimensional Scaling), PCO (Principal component Analysis) and RDA (redundancy analysis)

The multidimensional scaling (MDS) ordination method, to visualize the spatial responses of environmental parameters, is described in Figure 7. Points on the MDS with greatest separation represent parameters with greatest differences in environmental parameters. The patterns in environmental parameters structure, for each climatic coastal area, and the similarities to each other are represented based upon dissimilarities, while it is evident that there are substantial differences in environmental parameters among climate coastal areas (MDS 3D stress 0.2).

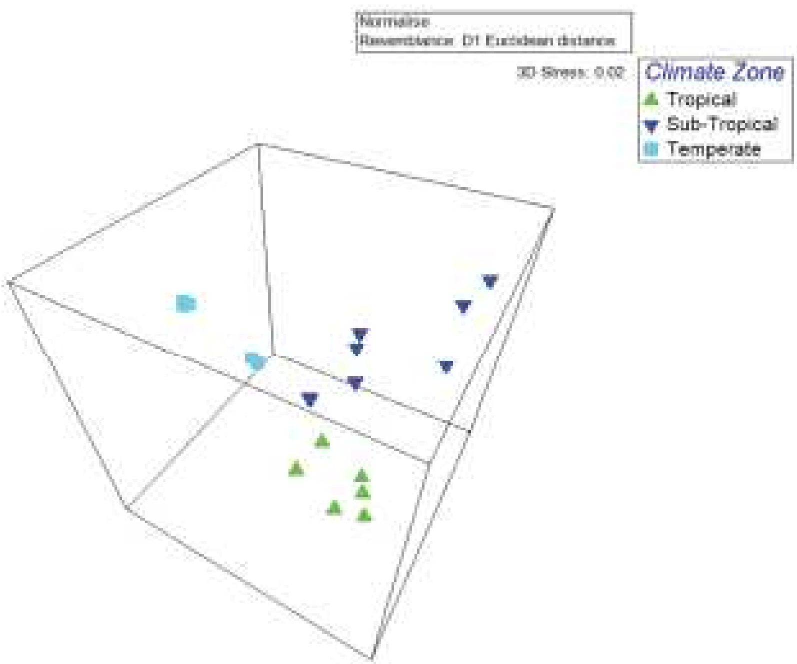


Figure 7. MDS ordination plot based on Euclidian distances between samples, arranged by studied climate areas.

The spatial variations and comparisons of environmental parameters by Principal Component Analysis (PCO) using a Permutational Multivariate Analysis of Variance (PERMANOVA) are described in Figure 8.

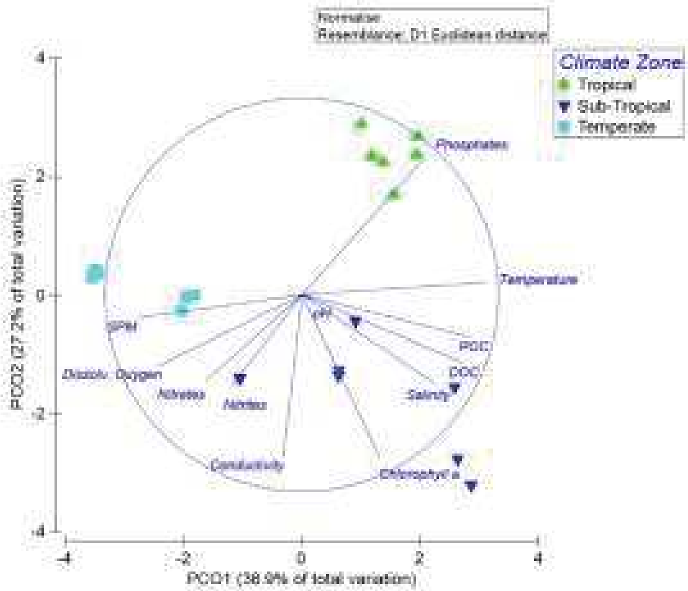


Figure 8. Principal Coordinate Analysis (PCO) scaling plot of the selected water parameters responses in the three climate zones. Parameters vectors are also overlapping the scaling plot with a Pearson correlation of 0.2.

Data points represent the structure of environmental parameters from replicate exclusion areas in each climate coastal area. The first two axes accounted for 74.1% of overall data variability. Overall results suggest Phosphates as the main discriminant environmental descriptor for Tropical areas. The Temperature, Salinity, Conductivity, Nitrates, Nitrites, POC and DOC were the main descriptors for the sub -Tropical areas. SPM, Dissolved Oxygen and nitrites were the main descriptors for the temperate areas. The biological and environmental data analyzed by redundancy analysis (RDA), as environmental descriptors are shown in a triplot ordination diagram (Figure 9). The first two axes accounted for 96.9% of overall data canonical variability. Both axis were associated to spatial variability. The first axis was strongly characterized by the opposition between chlorophyll *a*, with higher levels in subtropical areas, and SPM and phosphates, with higher concentrations in temperate and tropical locations. This axis is also characterized by a clear separation of subtropical from tropical and temperate water conditions. The second axis was characterized by the opposition between temperate and tropical conditions. Overall, both axes separated clearly the three selected areas.

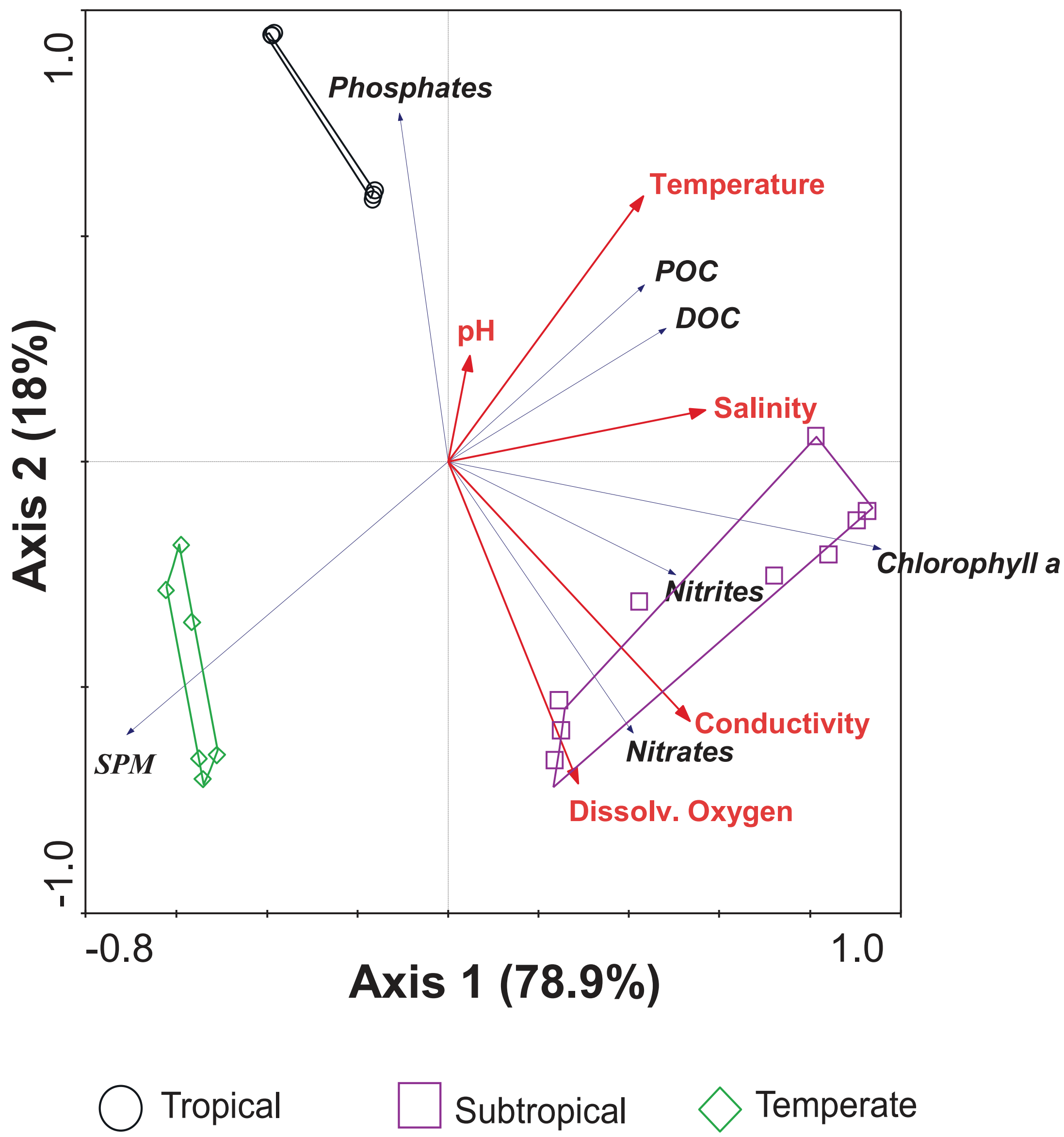


Figure 9. Redundancy analysis (RDA) ordination diagram with water parameters measure in the three selected climate areas. The water temperature, salinity, dissolved oxygen conductivity and pH selected as environmental descriptors and nitrates, nitrites, phosphates, chlorophyll *a*, POC, DOC and SPM levels as biological descriptors. First axis is horizontal, second axis is vertical; both axes explained 96.9% of total variability.

DISCUSSION

Climate driven changes in coastal areas nutrients, temperature and carbon/nitrogen/phosphates cycles parameters play a key role in the global biogeochemical cycles by linking terrestrial, oceanic and atmospheric reservoirs (Walsh, 1991). Since these systems represent a major pathway in the global carbon cycle, it is important to investigate the concentration and flux of the forms of carbon being transported out of these systems. Understanding dissolved (DOC) and particulate organic carbon (POC) losses and their impact on carbon budgets is also essential to meet the carbon sequestration targets in light of potential future climate changes (Pawson *et al.*, 2008). Although, the characterization of carbon dynamics, its decomposition into carbon dioxide and lost to the atmosphere through the coastal continuum of rivers, estuaries, marshes, and continental shelves before reaching and the open ocean is still controversial (Cai, 2011). Tropical coastal areas usually present several differences from temperate coastal areas, including the higher rainfall rates, the presence of perennial vegetation that reduces the soil erosion rates and a limited temperature variation. Besides that, the rivers from these regions present high water discharge, transport a smaller amount of suspended particulate matter (mainly due to the vegetation presents a higher density) and present a higher nutrient concentration (Meybeck, 1982; Nittrouer *et al*, 1995; Zhang, 1999). In temperate areas, physicochemical and biotic conditions usually display clear temperature dependent seasonal patterns (Drake and Arias 1991; Childers *et al*. 2006 a).

Climate conditions are expected to influence organic matter derived from primary production through changes to precipitation, fresh water delivery, and stratification, leading to changes in nutrient delivery, primary production, and primary producer species composition (Doney 2006, El-Najjar *et al*. 2010, Paerl *et al*. 2010, Rabalais *et al*. 2010). Alterations of precipitation patterns are also expected to change the magnitude and timing of freshwater delivery, thereby influencing the delivery of dissolved and particulate forms of organic matter from the surrounding watershed (e.g., allochthonous sources such as soils, vegetation detritus, and marsh export). Changes in freshwater delivery also have the potential to influence residence time and environmental conditions (e.g., light, stratification, microbial communities) under which organic matter is processed within coastal areas (Paerl *et al*., 2006 c, Rabalais *et al*., 2009, Whitehead *et al*., 2009).

Temperature and salinity are important climatic parameters due to its influence in the suspended particle aggregation and water quality (Gleick, 2003). Water temperature impacts water quality directly, increasing water temperature, or indirectly, reducing water volumes. Rise in temperature usually affects the rate of biogeochemical processes and a change in flow volumes alters residence times and dilution. In the case of VPA low salinity values could be related to the vicinity to Ponte de Lima River influence. The patterns of temperature variation in this study were the most expected and predictable, due to the location of sampling sites in three different climatic regions, and climate has a direct influence on water temperature, mainly determined by the air temperature (Sarmiento *et al*, 2004, Steinacher *et al*, 2010). Salinity variations are also important due to its influence on the flocculation/ aggregation, and hence the settling velocity, of the suspended particles (SPM), the higher the salinity, the greater the aggregation of suspended particles, the bigger the flocs and the faster the settling velocity (Syvitski & Milliman, 2007, Milliman & Farnsworth. 2011). The low salinity values relate to the proximity to estuarine mixing zones, because the diurnal evolution of salinity and temperature generally varied with tidal influence.

Warmer water tends to have lower levels of dissolved oxygen. Low water velocities promote lower turbulence which results in lower dissolved oxygen with effects on water quality. In these conditions, the BOD and NH4+ will increase while the dissolved oxygen will decrease (Mimikou *et al*., 2000). The physical unstable layer, where settling and resuspension of particles are intense, varied on the tidal time scale. This supposes that, at these concentrations, the turbulent diffusion of oxygen values compensates the biological oxygen demand (that increases when latitudinal increases). Oxygen consumption can be classified into three components: (1) water-column respiration, which increases with SPM concentrations; (2) sediment respiration; and (3) resuspension processes, which include a dilution term due to the input of anoxic pore water and a consumption term due to the oxidation of organic and inorganic species produced in the fluid mud (e.g., DOC, NH , and Mn). The latter component is important because oxygen deficits in surface waters can occur after resuspension events (Parker *et al*. 1994; Thouvenin *et al*. 1994). These results could be related to Temperature, Salinity and Dissolved Oxygen significant differences observed in these coastal areas, important climatic parameters that participates in the aggregation of suspended particles and water quality. Primary production is strongly limited by light availability and suspended particulate matter (SPM). This mainly affects photosynthesis and water temperature. Under non-nutrient limiting conditions, light availability is often the predominant factor in determining Chl *a* variation in coastal waters (Pennock, 1985). The last two phenomena are directly linked to climate change (Guo *et al*. 2009). Other factors tested such as nitrification rate is a temperature dependent process, corresponding to higher temperatures to lower nitrate concentrations. However, this might be overwhelmed by the changes in the dilution and residence time associated with changes in river discharge (Walling & Webb, 1992; Arnell, 1998; Mimikou *et al*., 2000).

Some results obtained in this research indicate “hypoxia” values on the tropical and temperate sampling data. It is well known that generally nutrient enrichment is directly or indirectly related to hypoxia and anoxia in the marine environment (Kramer, 1994). This might be related to the sampling collections at low tide, while the nutrient concentration were affected by the internal or external factors relating the nutrient cycling’s. A coastal area can receive external nutrient supplies (including nutrient elements fixed in organic material) by advection (adjacent areas), from land runoff (via rivers, coastal runoff and direct discharge) and from the atmosphere (or via changes in nitrogen fixation (Radach *et al*. 1990 b). Along with precipitation and runoff, materials transported from land via tributaries include soils, the remains of plant and animal matter, the products of soil degradation and respiration, natural and human-applied nutrients, contaminants, and other materials. The underlying rocks, soils, topography, vegetation and land use also have an important impact on delivery of these materials. The input of contaminants from diffuse sources, in particular from agricultural production and human activities, using extra nutrients and pesticides, and precipitation patterns may promote delivery of carbon, organic matter and other materials to coastal waters. Organic carbon losses resultant from the microbial decomposition processes, in anoxic environments, increase nitrites, nitrates and sulfates inputs (Valiela, 1995) and in nitrogen cycles dominated by a gaseous phase and microbial transformation involving changes in the oxidation state cause by denitrification, consisting of NO^{3-} transformation to NO^{2-} and requires a supply of organic compounds. The importance of this is that some nitrate is produced even in sediments or water with low oxygen content (Valiela, 1995), being the light nitrogen fixation energy source (Dugdale *et al*., 1961). Polyphosphates traces are found naturally in seawater and high concentrations of polyphosphates are often used as an indication of pollution in waste waters. Uptake by primary producers and bacteria is responsible for the low phosphate concentration typical of surface waters. The phosphates significant differences obtained in the present work might be due to the spatial variations (distinct climatic zones). Some studies suggested that phosphorous regeneration has a strong relation with bathymetry, where in coastal areas the renewable is fewer than 70%, less than in oceanic waters that could be near of 99%. Oxygen uptake by organisms in water correlates to phosphorus (and nitrogen) concentrations, since nutrients are released during aerobic respiration of organic matter (Valiela, 1995). The obtained results showed that the relative supply of N versus P may be sensitive to climate and circulation relating to changes in the rate of fixed-nitrogen removed by denitrification (Ganeshram *et al*, 1995) which promotes high dynamic oxic/anoxic zones (Gwenaël *et al*, 1999).

The solar and temperature influence are major factors in the control of carbon balance, mainly due to biological activity and daily (light/dark) variability (Bozec *et al*., 2012). The net role of terrestrial loadings on coastal areas metabolism is also an important factor in this process, mainly on total organic carbon loading to the ocean as a heterotrophic state (Cai, 2011). On the other hand, tidal influence is also very important not only for hydrographic parameters but also for dissolved and particulate organic carbon, nutrients and suspended particulate matter, because tides have substantial effects on carbon sequestration (Ribas-Ribas *et al*., 2011a) and low tides anoxic conditions could contribute to DOC low levels (Gwenaël *et al*, 1999). The high values of POC and DOC obtained in this work showed the importance of the strong influence of estuarine proximity and river discharges, industrial effluents, agricultural runoff, and domestic sewage discharged into the surface waters, in each sampling site~~s~~ in the three climatic regions, resulting in increased levels of POC and DOC. Although, the internal factors might also contribute to the POC/DOC increase relating to the release (exudation) by producers and excreted cycles by consumers, such as increase the discharges of organic matter by the different sources (Valiela, 1995). The results also showed the important inputs of suspended particulate matter (SPM) and dissolved organic matter mainly from the ocean probably because the sampling sites of all climatic zones were located under higher tidal movements influence, increasing the suspension matter in these places. The SPM regulates the partition coefficient, and hence also the two major transport routes, the dissolved transport in the water (the pelagic route) and the particulate sedimentation (or benthic) route, of all types of materials and contaminants. The suspended particulate matter is capable of transporting and releasing loads of adsorbed nutrients, pesticides, heavy metals, and other toxins as well as reducing the light penetration into the water. Further studies of the transport organic carbon, mainly through rivers and the atmosphere is an important indication of understanding the process in the global carbon cycle, because the organic matter presented in particulate or dissolved form, is used as a source of energy, nutrition, formation of fossil deposits and environmental conditions. Although, careful consideration of riverine, coastal and mudflat contributions must still be given in developing considerations to describe the ecological significance of particle dynamics in coastal areas, because sinking and suspending particle populations are dynamic entities that the material is continuously transformed and exchanged between them (Jago *et al*., 2006). However, In situ primary production, principally from phytoplankton, can be added to the particulate organic matter (Håkanson *et al*., 2004), and tidal runoff and erosion from intertidal mudflats as an important contributor of dissolved organic matter and SPM (Håkanson & Eckhéll, 2005).

The coastal areas physic-chemical characterization and carbon, nitrogen and phosphates variations in three climatic scenarios described here reveal a large input of nutrients, POC, DOC and Chlorophyll *a*., compared with other studies reported in the Atlantic and continental water (Smith and Hollibaugh, 1993; Bianchi *et al*, 1993; Alongi, 1995; Ganeshram et, al, 1995, Gattuso *et al*, 1998, Biondi *et al*, 2001, Brunskill, 2009, d'Orgeville & Peltier, 2007, Passow & Carlson. 2012.). The results showed the influence of climatic patterns of the physic-chemical variations, mainly temperature and salinity, on the other factors in each climatic zone, and suggested that specific characteristics of SPM in each location were highly contributed by sedimentary materials and could have been a rather consistent influence on physic-chemical processes, mainly as a source or sink of carbon (POC, DOC) and nutrients. On the other hand, the particulate organic carbon (POC) pool in the coastal waters, which includes autotrophic and heterotrophic organisms as well as biogenic detrital particles, is a highly dynamic carbon pool, representing one carbon reservoir of substantial interest and its variability has been yet poorly characterized. The mechanisms of SPM increase, such as by natural disturbance leading to resuspension, can facilitate future research to discriminate the impacts of increasing anthropogenic inputs on SPM dynamic. The present work investigates some correlations between factors, such as temperature influence (spatial variation) in other physic chemical factors, and also negative values were found in tropical and subtropical sample data. This is due to the sampling sites situated in strictly estuarine zones correlation with marine sampling locations, receiving the freshwater influence at low tide collections.

The MDS and PCO results, overall, suggest Phosphates as the main discriminant environmental descriptor for Tropical areas. The Temperature, Salinity, Conductivity, Nitrates, Nitrites, POC and DOC were the main descriptors for the sub -Tropical areas. SPM, Dissolved Oxygen and nitrites were the main descriptors for the temperate areas. The results of RDA in a triplot ordination diagram, also showed that the levels of chlorophyll *a*, associated to water salinity, phosphates and SPM were the main discriminative measured descriptors, with a major contribution on the differentiation of sub-Tropical areas from the other two selected zones. The selected water parameters were able to discriminate the three climate areas.

The effects of climate-driven conditions in nutrient dynamics and organic material processing are determinant for the success of management strategies on the structure and functioning of coastal ecosystems. Sustainable managing and protecting coastal wetlands and marine ecosystems for their carbon value will generate significant co-benefits by reducing degradation and promoting the restoration and sustainable management of coastal wetlands and marine ecosystems. This reinforces socio-ecological resilience and reduces vulnerability to climate change impacts. Following the conclusions and recommendations of The United Nation Framework Convention on Climate Change (UNFCCC, 2010, IPCC, 2011) advancing nature-based mitigation using coastal wetlands and marine ecosystems requires a range of priority actions, including: " baseline data, monitoring and verification approaches, expansion of scientific understanding of large-scale GHG pathways through oceanic systems, and for coastal wetlands and near-shore marine ecosystems" (UNEP, 2006, IPCC, 2007, Crooks *et al*, 2010, 2011). These data can then be used in ecosystem level models to predict the effects of climate change on marine and coastal habitats and potentially to evaluate the effects of different management scenarios. For the future is conditioned on decisive political action to manage environmental resources by ensuring both sustainable human progress and survival. Further water monitors and reports on the state and management of the marine coastal water resources through more exhaustive scientific evidence works are needed to provide a more in depth analysis of a specific issue or geographic areas. Managers and policy makers will therefore need to consider the close interplay between land-use, climate change and the coastal waters characteristics in the development and implementation of management strategies for regulating overall coastal water and environmental quality.

CONCLUSION

The local atmospheric and hydrodynamics processes interacting in complex ways influenced the physical and chemical attributes of the water column that regulates biological productivity and community structure. The availability and cycling of nutrients were determined by an interaction of physical, chemical, biological and climatic processes in each ecosystem. This interaction of processes is important to determine the flow of nutrient release, with specific pathways for the transfer of particulate and dissolved organic matter patterns and ultimate fate of nutrients in each system. The effects of climate-driven conditions in nutrient dynamics and organic material processing are determinant for the success of management strategies on the structure and functioning of coastal ecosystems. The obtained data can be used in ecosystem level models to predict the effects of climate change on marine and coastal habitats and potentially to evaluate the effects of different management scenarios.

The organic matter pool was a highly dynamic carbon pool that represents one carbon reservoir of substantial interest. The mechanisms of organic matter and nutrient relationships, such as a natural disturbance leading to resuspension, need further research to discriminate the impacts of increasing anthropogenic inputs on biogeochemical dynamic. The levels of chlorophyll a, associated to water salinity, phosphates and SPM were the main discriminative measured descriptors, with a major contribution on the differentiation of subtropical areas from the other two selected zones. The selected water parameters were able to discriminate the three climate areas. Overall, despite the relatively high environmental and anthropogenic influences, effective processing of different sources and forms of nutrients and organic matter was observed, which significantly reduces their signatures. Most of these materials were largely transformed and decreased in amount probably because of flocculation and removal of sediment, microbial degradation and/or dilution by other organic matter sources prior to export to the coastal ocean.

For the future is conditioned on decisive political action to manage environmental resources by ensuring both sustainable human progress and survival. Furthermore, water monitors and reports on the state and management of the marine coastal water resources through more exhaustive scientific evidence works are needed to provide a more in depth analysis of a specific issue or geographic areas. Managers and policy makers will therefore need to consider the close interplay between land-use, climate change and the coastal waters characteristics in the development and implementation of management strategies for regulating overall coastal water and environmental quality.

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CHAPTER 3

Chemical Stressors Bioaccumulation on Natural Populations

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Organochlorine pesticides, PCBs and PAHs in sediment and natural populations of sea anemones from three geographical regions

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INTRODUCTION

Pollution, destruction of sensitive coastal habitats, threats to aquatic biodiversity and significant socio-economic costs are the more important coastal areas impacts on environment and can involve health risks. Ecological effects from chemical stressors are strongly dependent on exposure characteristics. A wide range of compounds, persistent organic pollutants (POPs) including organochlorinated pesticides (OCPs), polychlorinated byphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) enter marine ecosystems (reef systems) through various pathways and affect different reef species and/or life history stages (Van Dam *et al.*, 2011).

Persistent organic pollutants were used intensively around the world and are ubiquitous environmental contaminants that once released may remain in the environment for a long time and undergo long-range transportation (Sun et al., 2009).

Organochlorinated pesticides (OCPs) were intensively used around the world to control pest and disease vectors. The main OCPs used were DDT, however the use of endosulfan and gamma-hexachlorohexane (lindane) are still in use mainly in developing countries (Ockenden et al., 2003). Due to their non-flammability, chemical stability, high boiling point, and electrical insulating properties, PCBs were used in hundreds of industrial and commercial applications including electrical, heat transfer, and hydraulic equipment. As many POPs, once in the environment, PCBs do not readily break down and therefore may remain for long periods of time cycling between the different environmental compartments. As a consequence, PCBs are found all over the world. PCBs have been demonstrated to cause cancer, as well as a variety of other adverse health effects on the immune system, reproductive system, nervous system, and endocrine system (Ross, 2004).

PAHs are generated during the combustion processes and, in urban environment, emitted primarily by anthropogenic sources, such as vehicle emissions, coal and fossil fuel powered generation, petroleum refining, straw and firewood burning, industrial processing, chemical manufacturing, oil spills and coal tars (Masih and Taneja, 2006). PAHs tend to accumulate in soils as they are sparingly soluble, readily absorbable by soil particles, and difficult to be degraded (Ping et al., 2007). PAHs have become a major type of pollutant of urban areas (Agarwal, 2009) and present potential carcinogenic risks to urban residents (Szabová et al., 2008).

Chemical substances as POPs can be meaningfully ranked or classified according to their persistence (P), bioaccumulation (B), toxicity (T), and potential for long-range transport (LRT) only if these attributes can be shown to be intensive, as distinct from extensive, properties of the substance, i.e., they are independent of quantity of substance. POPs are known to be highly mobile, traveling long distances even to the remote corner of the Earth (Koziol and Pudykiewicz 2001; Wania and Mackay 1996). They exhibit a process known as the "grasshopper effect", in which these chemicals go through cycles of volatilizations and condensations.

According to Shiedeck et al (2007), the contaminant range direction has a strong tendency to follow the tropical to temperate latitudes (hot to cool latitudinal sites). In low latitudinal places, the tendency is to high evaporation and low deposition, in opposite to this, the high latitudes, the depositions levels is higher than evaporation

POPs are hydrophobic and lipophilic — i.e., they are fatsoluble while resisting breakdown in water. Their lipophilic tendency enables them to concentrate in fatty tissues of organism and bioaccumulate up in the food chain. As noted by Eckley (2001), the levels of POPs detected in organisms that are high on the trophic levels — such as seals, polar bear, predatory birds, mammals, and humans, are sometimes thousands of times higher than levels found in the immediate surroundings (Wania and Mackay 1999). Biomagnification is an increase in the concentration of POPs and other organochlorinated chemicals in organisms as they pass through the food chain.

Accumulation through food can result in concentrations in the consumer that are higher than predicted based solely on lipid–water partition ratios and greater than those in food (termed biomagnification) (Thomann and Connolly, 1984). Mechanisms, including bioconcentration or trophic transfer, and factors such as lipid content or trophic level, which influence POP concentrations in small aquatic organisms, such as zooplankton, are more poorly understood (Fisk et al., 2001b). Although a review of more than 5000 bioconcentration factor (BCF) reported in the literature finds that 45% of BCF values are subject to at least one major source of uncertainty and that measurement errors generally result in an underestimation of actual BCF values (Arnot and Gobas, 2006).

In the present study, bioaccumulations levels it was evaluated to quantify the chemical stressors levels on sea anemones natural populations. The species studied represent a wide range and cosmopolitan species, ecologically representative, sensitive and good key to answer environmental questions and modifications caused by namelly antropogenic manipulated environment. The ecological niches of these species is very importante to distinguish until the effect observed it is caused by stressor, because the one of they, could be linked to the representant of symbiotic organisms (zooxanthellae).

As coral symbiosis based upon algal primary production is the engine driving coral reef ecosystems, stressors that interfere with photosynthetic processes could undermine the basis of this biologically and economically important marine habitat with serious consequences (Lesser and Farrell, 2004).

In general, benthic invertebrates are reported to be ideal assessment indicators because they are relatively non-mobile and thus tend to be representative of the area being sampled (Reynoldson et al., 1995 in Dolenec *et al*, 2007). The use of sea anemones derived from the fact that this species showed a large distribution. In other hand, they are known as non-selective suspension feeders that hosting abundant bacterial populations, which may have differential contaminants values, and high bioaccumulation values, that could show a better description of the overall impact on the environment due to the presence of enriched sources and many types of discharges.

The objectives of this study were 1) to determine residues of OC, PCBs and PAHs in sediment and anemones from three climatic regions, 2) to identify the main chemicals groups are the most frequent, 3) to determine a probable bioconcentration factor (BCF), and 4) to establish a possible geographical pattern of POPs distribution,

MATERIAL AND METHODS

Study Area

To undertake an integrated monitoring strategy seven sampling sites were selected in the north, south and western Atlantic coast under the influence of different climatic environments and types of contamination (Fig. 1). The study locations comprehend reference and contaminated areas under antropogenic, industrial and harbour influence combining exposed and sheltered habitats. Figure 1 shows the locations of the sampling sites and table 1 indicate the geographical coordinates of each sampling sites.

1. Tropical environment

The study was undertaken in Itamaraca, a tropical tidal estuarine system (Figure l), located in Pernambuco in northeastern Brazil, 35 km north of Recife. It is a natural and under low anthropogenic influence environmental area (situated in APA Environment protection area) (Medeiros and Kjerfve, 1993; 2005). The other study area in the tropical environment was Olinda – Northeast coast Brazil the Casa Caiada - Rio Doce beach complex, a 4.5 km-long sandy coastline located at the northern end of Olinda City (Pernambuco, NE Brazil) (Fig. 1). The characteristics of this stressed coastal area were determined, first by those of the surrounding coastal sea and second, by the strong influence of urban activities (Pereira et al. 2003a).

Sampling sites (Brazil)

In southern tropical environmental Itamaracá (ITA- reference) (Pernambuco, Brazil) was selected as reference station and Olinda (OLI- contaminated) a displayed contaminated area (Pernambuco, Brazil), described as suffering the influence of anthropogenic and urban and/or industrial contamination pressures (Cerqueira and Pio, 1999). According to climatic scenarios, pollution sources and ecological characterizations, we classified all points, for these sample site, the follow abbreviations used it was: TROP/REF or CONT, that is meaning tropical place reference or contaminated place.

2. Sub –Tropical environment

The study area extends in the Itaipú bay (Niterói, Rio de Janeiro State) (southern coast Brazil) near the Imbuí Point on the west to the Itaipú Point on the east, containing the Piratininga, Camboinhas and Itaipú beaches, and its seaward limit corresponds to the aligned islands of Pai, Mãe, and Menina (Fig. 1).. Despite the enormous growth of population along the shoreline in Niterói, the water depth varies from a minimum of about 3-4 m (just seaward of the average breaking wave zone) to a maximum of 28 m, all of these depths pertaining to the shoreface environment (DHN 1974; ECP 1979, in: Lavenère-Wanderley 1999). The other studied area was the coastline Gulf of México, eastern part of Campeche (México).

Sampling sites (Brazil and Mexico)

In the southern sub-tropical environment three locals were selected, Punta Xen (PXE-reference) and Siho Playa (SHP-contaminated displayed) (Campeche, Mexico) and Itaipu (NIT- contaminated exposed) (Rio de Janeiro, Brazil) (Figure 1, Table 1). The NIT point were located the sampling stations with agricultural influence (Aa), Harbour influences and anthropogenic influence this has been described as a heavy metals polluted site (Monterroso et al., 2005). According to climatic scenarios, pollution sources and ecological characterizations, we classified all points, for these sample sites, the follow abbreviations were used SUBTROP/REF or CONT/ DIS or EXP, that is meaning subtropical place reference or contaminated, displayed or exposed place, respectively.

3. Temperate environment

For the temperate environment the study was carried on the Portuguese coast that is divided in three main regions: North, Centre and South. This division was made considering that Portugal is the southern geographical limit for many boreal species and the northern or western limit of subtropical and Mediterranean species (Saldanha, 1974).

Sampling sites (Portugal)

Two sites were selected in northwest temperate environment (Portugal), Vila Praia de Âncora, reference station (VPA-reference), Praia Norte (PN-contaminated). Concerning the sampling stations located along the NW Portuguese coast: VPA – Vila Praia de Âncora and PN-Praia Norte**.** VPA is located near small fishery villages and far from big population aggregates and potential sources of contamination (Agricultural, Urban and Harbour). Several studies performed in this coast indicated that this site is relatively undisturbed by anthropogenic pressures (Moreira et al., 2004; Moreira and Guilhermino, 2005). PN is located in the vicinity of important industrial facilities, namely an oil refinery and a harbour supporting intensive vessel traffic; thus, they are chronically exposed to petroleum-derived hydrocarbons, including PAHs and heavy metals (Leal et al., 1997; Salgado and Serra, 2001; Serra, 1998). According to climatic scenarios, pollution sources and ecological characterizations, we classified all points, for these sample site, the follow abbreviations used it was: TEMP/REF or CONT that is meaning temperate place reference or contaminated places, respectively.

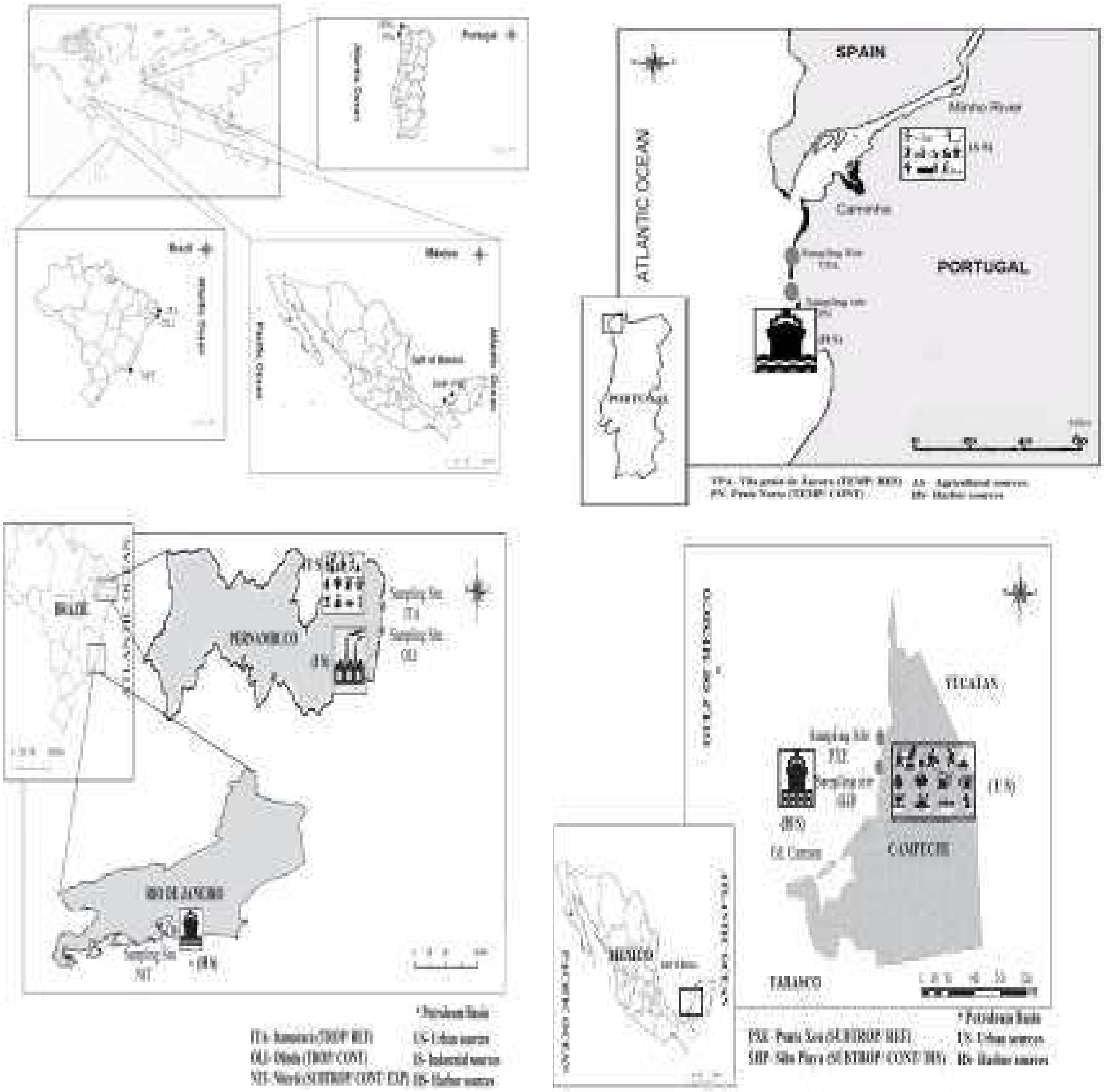

Figure. 1. Location of the sampling sites in the three climatic environments. Northwest Portuguese coast. Vila Praia de Âncora (VPA-reference; Viana do Castelo Praia Norte (PN)-urban and industrial effluents. Brazilian coast, Itamaracá (ITA)- reference, Olinda (OLI)I-urban and industrial effluents. Mexican coast, Punta Xen (PXE)-reference, Siho Playa (SHP) urban and industrial effluents.

Target species

Six target species were selected according their ecological importance, distribution and abundance. For the tropical environment it were selected *Anemonia sargassensis* (Hargitt, 1908) and *Bunodossoma cangicum* that are the most abundant sea anemones of Brazilian coast (Zamponi, 1998), with large distribution in media and infra littoral habitats (Corrêa 1964; Belém & Preslercravo, 1973; Dube, 1974; Gomes, 1996; Gomes and Mayal, 1997; Amaral et al., 2002). For the temperate environment it was selected *Actinia equina* (Linnaeus, 1758) and *Anemonia sulcata* (Forskal, 1775) that are the most representative species found on rocky shores of the European coast and as far as the coast of West Africa (Its range extends throughout the Atlantic coasts of Europe (Gadelha et al, 2010 and Gadelha et al, 2012), North Africa and into the Mediterranean and South Africa. For the subtropical environment were selected two representative species, *Actinia bermudensis* and *Bunodossoma caissarum,* common on rocks just below the low tide line, that presents large distributions occurred since the West Indies, Bermuda and northern Florida and South to Brazil (Belém and Preslercravo, 1973, Schlenz 1983; Ruppert and Fox 1988) (Table 1).

Table 1. Species, sampling sites with locations and coordinates information of each climatic scenarios.

Species	**Sampling Sites**	**Location**	**Coordinates**	**Climatic Environment**
Actinia equina, Anemonia sulcata	Vila Praia de Âncora (VPA)	Northwest, Portugal	41°49'13.54"N 8°52'26.05"W	Temperate
Actinia equina, Anemonia sulcata	Viana do Castelo Praia Norte (PN)	Northwest, Portugal	41°41'41.61"N 8°51'6.43"W	Temperate
Anemonia sargassensis, Bunodossoma cangicum	Itamaracá (ITA)	Recife, Brazil	7º47'S, 34º50'W 7º47'S, 34º50'W	Tropical Tropical
Anemonia sargassenssis	Olinda (OLI)	Recife, Brazil	7°58'27.21"S 34°49'54.46"W	Tropical
Actinia bermudensis	Punta Xen (PXE)	Campeche, Mexico	43° 2'56.80"W 19°20'8.91"N	Sub-Tropical
Actinia bermudensis	Siho Playa (SHP)	Campeche, Mexico	90°43'37.92"W 19°33'24.10"N	Sub-Tropical
Actinia bermudensis, Bunodosoma caissarum	Itaipu (NIT)	Rio de Janeiro, Brazil	22°58'18.10"S 43° 2'56.80"W	Su-Tropical

Animal and sediment sampling

Twenty sea anemones were collected (May to September 2012), at low tide, in the inter-tidal zone of the seven sampling sites (Fig. 1). The organisms collected were 1-3 cm in length. Samples were kept on ice during transport. From these, 20 animals of each species and sediment samples where used for bioaccumulations evaluation. Organisms were placed in thermally insulated boxes, previously filled with local water, and transported to the laboratory within 1–2 h of sampling. In each sampling site it was taken a sample of sediment. All samples were transported under cool temperature conditions. Sediments samples were dry in an oven at 40ºC for 48h. To determine contaminants on sediments, they was screening to 3 distinct fractions (<63µm; >63µm<1000µm and >2000µm).

Persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAHs) analysis

POPs and PAHs extraction in sediments and organisms was carried out according to the method proposed by Wang et al. (2007) with some little modifications. A CEM MARS Xpress Microwave Accelerated Reaction

System (CEM Corporation, Matthews, NC, USA) was used. In this work, portions of 5 g sediment were weighed into 55 mL perfluoroalkoxy (PFA) polymer extraction vessels equipped with Teflon-sealed lip-tight caps and polyetheretherketone (PEEK)-liners. In the case of anemones a pool of five anemones per sampling site were used. Microwave power was 1200 W (100%). The extraction solvent was 25 mL n-hexane and acetone (1:1, v/v). The extraction was performed in temperature-controlled mode. The extraction temperature was 110 °C and programmed as follows: ramp to 110 °C for 10 min, holding at 110 °C for 10 min. After the extraction completed, sediment/anemones were separated from solvent by filtration and the solvent was decanted into a pear-shaped flask.

The organic extracts obtained were evaporated to nearly dryness under reduced pressure in a 35 °C water bath using a rotary evaporator and evaporated to a small volume (about 1 mL). The concentrated extracts and two 2-mL portions of n-hexane from rinsing the sample flask were transferred to top of a chromatography column (30 cm × 10 mm i.d.) filled with 10 g silica gel (100–200 mesh) to separate the PAHs, and POPs fraction from other interfering matters. The silica gel was wet-loaded as slurry in n-hexane and capped with a thin layer of absorbent cotton (extracted with DCM as samples) to prevent the gel from spilling, and approximately 2 cm length of anhydrous sodium sulfate was added in the top. The column was sequentially eluted with three fractions 20 mL of n-hexane, 20 mL of n-hexane: DCM (1:1; v/v) and 20 mL of DCM to produce fractions enriched PAHs and POPs at flow rate of ∼2 mL min−1.

The final volume was adjusted to 1 mL under a gentle stream of N_2, and then the sample was transferred to 1.5 mL vial for gas chromatography analysis. Each set of samples was accompanied by a complete blank and a spiked blank that was carried through the entire analytical scheme in identical conditions for all samples.

PAHs were analyzed by GC with a Varian 3800 equipped with a DB-5, 5% fenil Metilpolisiloxano column, 30m x 0.25mm and a Flame Ionization detector (FID). PAHs were identified by comparing their retention times with those from the aromatic analytical standards by Supelco 48743 according to the 16 priority PAHs from method EPA 610 (acenaphthene, acenaphthylene, anthracene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, dibenz[a,h]anthracene, fluoranthene, fluorene, indeno[1,2,3-cd]pyrene, naphthalene, phenanthrene, pyrene).

POPs were analyzed by GC with a Varian 3800 equipped with a SGE HT8-PCB (8% Phenyl (equiv) Polycarborane-siloxane), 60m x 0.25mm and a Ni63 electron capture detector (ECD). The temperature of the injector was 150 °C and of the detector was 300 °C. The oven temperature was maintained at 60 °C/min and then reached to 320 °C with increases of 2 °C/min for 5 min. The nitrogen flux for the column was 2mL/min and a make-up of 30 mL/min. The qualitative data was obtained with the area calculated of the curve with the software Star Chromatography Workstation version 6 and the calibration standard.

For the OC identification and quantification a mix of standards were used: α, β and γ-HCH, heptachlor, aldrin, heptachlor epoxide, endosulfan I, dieldrin, *p,p* DDE, endrin, endosulfan II, endrin aldehyde, *p,p* DDD, endosulfan sulfate and *p,p* DDT (SUPELCO® 47426-U CLP Organochlorine Pesticide Mix); for the PCB the congeners standard used were: 28, 31, 44, 52,101,118, 138, 149, 153,180, 194 (SUPELCO® 47927 CEN PCB Congener Mix-1). The results is expressed in units of ug/g.

Quality assurance

Laboratory blanks were analyzed for quality assurance. Soil from a garden near to the University building and fish samples were used by triplicate. For POPs and PAHs analysis, 25 ng of 2, 4, 5 trichorobiphenyl (TCB) were added to each sample as an internal standard before the extraction, they were refrigerated for 48 hours. One of the subsamples was not spiked with the standard in order to have a positive blank. Afterward they were extracted and processed in an identical manner with the rest of the samples.

Data analysis

Bioconcentrations Factors

The bioconcentration factor (BCF) is defined as the ratio of the chemical concentration in an organism CB, to the total chemical concentration in the water CWT, or in this study, in sediments (MacKay and Fraser, 2000). To integrate the biological factors with environmental, it was calculated the "Bioconcentrations factors" using the follow formula:

$$BCF = [\text{Organism contaminant concentration}] \div [\text{sediment contaminant concentration}]$$

Both concentrations values it was a summatory results of each contaminant group, for replicates.

Statistical Analyses

The normality of data was tested (Kolmogorov Smirnov normality test) and the homogeneity of variance was verified (Barlett's test). The levels of PAHs and POPs analysed in the organisms and sediments, collected from the three climate areas, were compared using a one-way analysis of variance (One-Way ANOVA). Tukey's test was applied if significant differences among species and different climate zones were detected by ANOVA (Zar, 1996).

The selected contaminants spatial and species variations were analysed by Principal Coordinate Analysis (PCO). The biological and environmental data were also analysed by redundancy analysis (RDA), using the levels of PAHs and POPs in sea anemones as biological descriptors and the same contaminants, in sediments, as environmental descriptors. With the exception of PCO and RDA, all statistical analysis were performed, using SPSS Statistics (vers. 17). PCO tests were performed using PRIMER with PERMANOVA+ software (PRIMER v6 & PERMANOVA+ v1, PRIMER-E Ltd.) The RDA analysis was performed using the software CANOCO 4.5 for Windows (Biometris, The Netherlands).

RESULTS

The results of the studied samples for the PAHs and POPs compounds are presented in Tables, 2, 3 and 4. The PAHs determined were 2 to 6 ring compounds (Table 2). The majority of the PAHs observed were 4 to 6-ring compounds and most predominantly present were Fluoranthene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Dibenz(a,h)anthracene, Benzo(g,h,i)perylene and Indeno(1,2,3-cd)pyrene (Table 2). The results show that the PAHs were present in high concentrations across all the sediments locations and anemones species tissues.

Table 2. Summary table with abbreviations and the number of rings from the 16 PAHs analysed in anemones tissues and sediments collected from the three climate zones.

PAH	Abbreviation	Number of Rings
Naphthalene	Na	2
Acenaphthylene	Ac	3
Acenaphthene	Ace	3
Anthracene	An	3
Fluorene	Fl	3
Phenanthrene	Phe	3
Fluoranthene	Flu	4
Pyrene	Py	4
Benzo(a)anthracene	BaA	4
Chrysene	Cr	4
Benzo(b)fluoranthene	BbF	5
Benzo(k)fluoranthene	BkF	5
Benzo(a)pyrene	BaP	5
Dibenz(a,h)anthracene	DBA	5
Benzo(g,h,i)perylene	BghiP	5
Indeno(1,2,3-cd)pyrene	IP	6

The highest concentrations for PAHs sea anemones tissues concentrations were registered in Tropical and Sub-tropical climate coastal areas. The results for PAHs sediments concentrations were much lower than levels reported for sea anemones tissues. The highest sediments concentrations were registered in temperate climate coastal areas (Table 3). The results for POPs sea anemones tissues concentration were much lower than PAHs levels reported. The results for POPs sediments concentrations were generally much higher than levels reported for sea anemones tissues. The highest concentrations were registered in Temperate and Tropical climate coastal areas. The majority of the PAHs observed were ∑HCHs, ∑DDT, ∑Endosulfan and ∑Drines (Table 3).

PAHs and POPs sea anemones tissues concentrations

In Tropical coastal areas the minimum PAHs sea anemones tissues concentrations (mean ± SEM) were found for Phenanthrene (1.59±0.000), and maximum for Indeno(1,2,3-cd)pyrene (309,61±56.08). It were also found high concentrations for Benzo(a)anthracene(120.70±108.08), Dibenz(a,h)anthracene (79,81±44.98), Benzo(g,h,i)perylene (53,22±9.807), Benzo(a)pyrene (22,93±6.301) and Chrysene (21.76±3.482). In Sub-Tropical coastal areas the minimum PAHs sea anemones tissues concentrations (mean ± SEM) were found for Naphthalene (0.96±0.224) and maximum for Indeno(1,2,3-cd)pyrene (1478.17±289.4). It were also found high concentrations for Dibenz(a,h)anthracene (411.35±90.51), Benzo(g,h,i)perylene (133.04±30.03), Pyrene (70.89±22.50) and Benzo(b)fluoranthene (32.05±19.17). In Temperate coastal areas the minimum PAHs sea anemones tissues concentrations (mean ± SEM) were found for Acenaphthene (0.14±0.024) and maximum for

Benzo(g,h,i)perylene (7.42±4.860). It were also found high concentrations for Indeno(1,2,3-cd)pyrene (2.93±0.880), Benzo(b)fluoranthene (2.71±1.065), Dibenz(a,h)anthracene (2.23±0.562) and Fluoranthene (2.10±0.443) (Table 3).

In Tropical coastal areas the minimum POPs sea anemones tisues concentrations (mean ± SEM) were found for ∑DDT (6.86±0.468) and maximum for ∑Drines (15.9±0.540). It was also found important concentrations for ∑HCHs (11.1±0.175) and ∑ Endosulfan (9.59±0.153). In Sub-Tropical coastal areas the minimum POPs sea anemones tissues concentrations (mean ± SEM) were found for ∑ Endosulfan (0.44±0.180) and maximum for ∑DDT (2.31±0.271). It was also found important concentrations for ∑HCHs (0.71±0.121) and ∑Drines (1.20±0.221). In Temperate coastal areas the minimum POPs sea anemones tissues concentrations (mean ± SEM) were found for ∑ Endosulfan (7.01±0.526) and maximum for ∑HCHs (13.4±0.637). It was also found important concentrations for ∑DDT (13.3±0.594) and ∑Drines (10.8±0.762) (Table 3).

PAHs and POPs sediments concentrations

In Tropical coastal areas the minimum PAHs sediments concentrations (mean ± SEM) were found for Pyrene (0.04±0.039) and maximum for Chrysene (0.29±0.269). It were also found important concentrations for Benzo (a) pyrene (0.20±0.132), Benzo (b) fluoranthene (0.19±0.120) and Benzo (k) fluoranthene (0.19±0.119). In Sub-Tropical coastal areas the minimum PAHs sediments concentrations (mean ± SEM) were found for Phenanthrene (0.02±0.023) and maximum for Benzo (a) pyrene (5.89±4.968). It were also found important concentrations for Chrysene (4.93±3.726), Pyrene (2.49±2.191), Indeno(1,2,3-cd)pyrene (1.64±0.716) and Benzo(g,h,i)perylene (1.21±0.795). In Temperate coastal areas the minimum PAHs sediments concentrations (mean ± SEM) were found for Pyrene (0.03±0.025) and maximum for Indeno(1,2,3-cd)pyrene (2.17±2.010). It were also found important concentrations for Benzo (a) pyrene (1.75±1.296) and Dibenzo (a, h) anthracene (0.61±0.572) (Table 3).

In Tropical coastal areas the minimum POPs sediments concentrations (mean ± SEM) were found for ∑HCHs (5.30±0.042) and maximum for Drines (15.9±0.54). It was also found important concentrations for ∑Drines (13.1 ±0.38) and ∑Endosulfan (12.03±0.40). In Sub-Tropical coastal areas the minimum POPs sediments concentrations (mean ± SEM) were found for ∑HCHs (4.57±3.221) and maximum for ∑DDT (37.3±19.61). It was also found important concentrations for ∑Drines (16.4±0.075) and ∑Endosulfan (9.60±5.167). In Temperate coastal areas the minimum POPs sediments concentrations (mean ± SEM) were found for ∑HCHs (5.30±0.042) and maximum for ∑DDT (58.5±0.655). It was also found important concentrations for ∑Drines (13.1±0.382) and ∑Endosulfan (12.3±0.407) (Table 3).

POPs and PAHs concentrations in selected species

A. sulcata

The values of the PAHs observed were generally very low in a similar pattern of *A. equina*. The minimum PAHs concentrations (mean ± SEM) for *A. sulcata* were found for Acenaphthene (0.12±6.030) and maximum for Benzo (g, h, i) perylene (13.50±10.511). It was also found important concentrations for Benzo (k) fluoranthene

(5.64±2.043), Indeno (1, 2, 3-cd) pyrene (3.12±1.078), Fluoranthene (3.25±0.766) and Benzo (b) fluoranthene (2.25±1.123) (Table 4).

The values of the POPs observed for *A. sulcata* were generally high. The minimum POPs concentrations (mean ± SEM) for *A. sulcata* were found for ∑Endosulfan (4.05±0.570) and maximum for ∑HCHs (19.9±0.832). It was also found important concentrations for ∑DDT (7.69±0.625) and ∑Drines (4.92±0.590) (Table 3).

B. caissarum

The values of the PAHs observed concerning to 4 to 6-ring compounds were very high in a similar pattern observed for *A. bermudensis* and *A. sargassensis*. The minimum PAHs concentrations (mean ± SEM) for *B. caissarum* were found for Naphthalene (1.16±0.300) and maximum for Indeno (1, 2, 3-cd) pyrene (751.93±310.8). It was also found important concentrations for Dibenz(a,h)anthracene (235.08±120.1), Benzo(g,h,i)perylene (72.17±33.75), Benzo(a)pyrene (15.77±3.831) and Benzo(k)fluoranthene (6.21±2.574) (Table 3).

The values of the POPs observed for *B. caissarum* were generally very low. The minimum POPs concentrations (mean ± SEM) for *B. caissarum* were found for ∑Endosulfan (0.26±0.000) and maximum for ∑DDT (1.70±0.000) (Table 3).

Table 3. Concentrations of PAHs and POPs measured in the anemones tissues and sediments from the three selected climate areas (ug/g). The indicated values are the mean ± SEM.

	Tissues			Sediments		
	Tropical	SubTropical	Temperate	Tropical	SubTropical	Temperate
PAHs						
Naphthalene	**4.03** ± 0.295	**0.96** ± 0.224	**0.24** ± 0.061	**0.00** ± 0.000	**0.00** ± 0.000	**0.00** ± 0.000
Acenaphthylene	**12.68** ± 0.000	**4.52** ± 1.629	**0.18** ± 0.023	**0.00** ± 0.000	**0.00** ± 0.000	**0.00** ± 0.000
Acenaphthene	**3.14** ± 0.379	**1.93** ± 0.614	**0.14** ± 0.024	**0.00** ± 0.000	**0.00** ± 0.000	**0.00** ± 0.000
Anthracene	**4.85** ± 1.546	**2.43** ± 0.716	**0.29** ± 0.052	**0.00** ± 0.000	**0.00** ± 0.000	**0.00** ± 0.000
Fluorene	**3.96** ± 0.531	**2.64** ± 0.690	**0.25** ± 0.075	**0.00** ± 0.000	**0.00** ± 0.000	**0.00** ± 0.000
Phenanthrene	**1.59** ± 0.000	**0.81** ± 0.267	**1.36** ± 0.472	**0.00** ± 0.000	**0.02** ± 0.023	**0.00** ± 0.000
Fluoranthene	**5.65** ± 0.672	**3.95** ± 1.222	**2.10** ± 0.443	**0.00** ± 0.000	**0.91** ± 0.586	**0.00** ± 0.000
Pyrene	**13.73** ± 4.731	**70.89** ± 22.50	**1.00** ± 0.209	**0.04** ± 0.039	**2.49** ± 2.191	**0.03** ± 0.025
Benzo(a)anthracene	**120.70** ± 108.8	**14.77** ± 3.986	**0.64** ± 0.227	**0.07** ± 0.060	**0.64** ± 0.397	**0.04** ± 0.040
Chrysene	**21.76** ± 3.482	**4.72** ± 0.905	**1.76** ± 0.361	**0.29** ± 0.269	**4.93** ± 3.726	**0.00** ± 0.000
Benzo(b)fluoranthene	**9.12** ± 1.759	**32.05** ± 19.17	**2.71** ± 1.065	**0.19** ± 0.120	**1.07** ± 0.678	**0.06** ± 0.061
Benzo(k)fluoranthene	**8.82** ± 2.412	**8.64** ± 2.364	**6.08** ± 1.481	**0.19** ± 0.119	**0.77** ± 0.616	**0.87** ± 0.594
Benzo(a)pyrene	**22.93** ± 6.301	**11.56** ± 1.971	**1.69** ± 0.361	**0.20** ± 0.132	**5.89** ± 4.968	**1.75** ± 1.296
Dibenz(a,h)anthracene	**79.81** ± 44.98	**411.35** ± 90.51	**2.23** ± 0.562	**0.00** ± 0.000	**0.39** ± 0.219	**0.61** ± 0.572
Benzo(g,h,i)perylene	**53.22** ± 9.807	**133.04** ± 30.03	**7.42** ± 4.860	**0.00** ± 0.000	**1.21** ± 0.795	**0.34** ± 0.325
Indeno(1,2,3-cd)pyrene	**309.61** ± 56.08	**1478.17** ± 289.4	**2.93** ± 0.880	**0.08** ± 0.052	**1.64** ± 0.716	**2.17** ± 2.010
POPs						
∑HCHs	**2.73** ± 0.121	**0.71** ± 0.121	**13.4** ± 0.637	**11.1** ± 0.175	**4.57** ± 3.221	**5.30** ± 0.042
∑DDT	**21.5** ± 0.224	**2.31** ± 0.271	**13.3** ± 0.594	**6.86** ± 0.468	**37.3** ± 19.61	**58.5** ± 0.655
∑Endosulfan	**2.03** ± 0.135	**0.44** ± 0.180	**7.01** ± 0.526	**9.59** ± 0.153	**9.60** ± 5.167	**12.3** ± 0.407
∑Drines	**1.96** ± 0.099	**1.20** ± 0.221	**10.8** ± 0.762	**15.9** ± 0.540	**16.4** ± 0.075	**13.1** ± 0.382

A. bermudensis

The values of the PAHs observed concerning to 4 to 6-ring compounds were very high. The minimum PAHs concentrations (mean ± SEM) for *A. bermudensis* were found for Naphthalene (1.34) and max for Indeno (1, 2, 3-cd) pyrene (1046±476.2). It was also found important concentrations for Dibenz(a,h)anthracene (444.33±457.7), Benzo(b)fluoranthene (83.40±70.45), Benzo(g,h,i)perylene (74.34±56.55) and Benzo(a)pyrene (11.64±2.204) (Table 4).

The values of the POPs observed for *A. bermudensis* were generally high. The minimum POPs concentrations (mean ± SEM) for *A. bermudensis* were found for ∑HCHs (0.81±0.000) and maximum for ∑DDT (4.66±0.000) (Table 4).

A. equina

The values of the PAHs observed were generally very low. The minimum PAHs concentrations (mean ± SEM) for *A. equina* were found for Naphthalene (0.11±0.000) and maximum for Benzo (k) fluoranthene (6.43±2.009). It was also found important concentrations for Dibenz(a,h)anthracene (3.08±1.685), Indeno(1,2,3-cd)pyrene (2.76±1.356), Benzo(g,h,i)perylene (2.56±0.711) and Dibenz(a,h)anthracene (2.50±0.937) (Table 4).

The values of the POPs observed for *A. bermudensis* were generally very high. The minimum POPs concentrations (mean ± SEM) for *A. equina* were found for ∑HCHs (6.89±0.879) and maximum for ∑DDT (19±0.338). It was also found important concentrations for ∑Drines (17.3±1.738) and ∑Endosulfan (9.97±1.213) (Table 4).

Actinia spp.

The values of the PAHs observed concerning to 4 to 6-ring compounds were very high in a similar pattern observed for *A. bermudensis*. The minimum PAHs concentrations (mean ± SEM) for *Actinia* spp**.** were found for Naphthalene (0.01±0.000) and maximum for Indeno (1, 2, 3-cd) pyrene (2073.35±423.4). It was also found important concentrations for Dibenz(a,h)anthracene (553.50±156.2), Benzo(g,h,i)perylene (217.17±47.52), Pyrene (178.70±34.34), Benzo(b)fluoranthene (32.49±18.71), Benzo(a)anthracene (30.26±6.975) and Benzo(k)fluoranthene (11.35±5.667) (Table 4).

The values of the POPs observed for *Actinia* spp. were generally very low. The minimum POPs concentrations (mean ± SEM) for *Actinia* spp. were found for ∑Endosulfan (0.01±0.041) and maximum for ∑Drines (1.78±0.497) (Table 4).

A. ***sargassensis***

The values of the PAHs observed were generally very high mainly concerning to 4 to 6-ring compounds. The minimum PAHs concentrations (mean ± SEM) for *A. sargassensis* were found for Phenanthrene (1.59±0.000) and maximum for Indeno (1, 2, 3-cd) pyrene (309.61±56.08). It was also found important concentrations for Benzo(a)anthracene (120.70±108.8), Dibenz(a,h)anthracene (79.81±44.98), Benzo(g,h,i)perylene (53.22±0.807), Benzo(a)pyrene (22.93±6.301), Chrysene (21.76±3.482), Pyrene (13.73±4.731) and Acenaphthylene (12.68±0.000) (Table 4).

The values of the POPs observed for *A. sargassensis* were generally low. The minimum POPs concentrations (mean ± SEM) for *A. sargassensis* were found for ∑Drines (1.96±0.099) and maximum for ∑DDT (21.5±0.224). It was also found important concentrations for ∑HCHs (2.73±0.121) and ∑Endosulfan (2.03±0.135) (Table 4).

Table 4. Selected species PAHs and POPs concentrations (ug/g). The values are the mean ± SEM.

	A. bermudensis	*A.equina*	*Actinia* spp.	*A. sargassensis*	*A. sulcata*	*B. caissarum*
PAHs						
Naphthalene	**1.34** ± 0.341	**0.11** ± 0.000	**0.01** ± 0.000	**4.34** ± 0.169	**0.36** ± 0.000	**1.16** ± 0.300
Acenaphthylene	**2.13** ± 0.625	**0.19** ± 0.005	**9.73** ± 4.675	**12.68** ± 0.000	**0.18** ± 0.038	**2.51** ± 0.613
Acenaphthene	**1.87** ± 0.709	**0.17** ± 0.019	**3.61** ± 1.800	**3.14** ± 0.379	**0.12** ± 0.030	**0.87** ± 0.256
Anthracene	**3.62** ± 1.403	**0.22** ± 0.034	**1.06** ± 0.739	**4.85** ± 1.546	**0.35** ± 0.080	**2.09** ± 0.912
Fluorene	**1.48** ± 0.397	**0.20** ± 0.037	**5.00** ± 1.298	**3.96** ± 0.531	**0.30** ± 0.128	**0.70** ± 0.196
Phenanthrene	**0.49** ± 0.135	**1.20** ± 0.372	**1.68** ± 0.633	**1.59** ± 0.000	**1.56** ± 0.951	**0.33** ± 0.055
Fluoranthene	**1.76** ± 0.315	**1.18** ± 0.263	**7.26** ± 2.742	**5.65** ± 0.672	**3.25** ± 0.766	**1.87** ± 0.461
Pyrene	**1.68** ± 0.823	**1.24** ± 0.325	**178.70** ± 34.39	**13.73** ± 4.731	**0.69** ± 0.187	**1.53** ± 0.827
Benzo(a)anthracene	**3.63** ± 1.249	**0.41** ± 0.095	**30.25** ± 6.975	**120.70** ± 108.8	**0.86** ± 0.430	**4.30** ± 0.946
Chrysene	**5.14** ± 1.135	**2.11** ± 0.490	**6.09** ± 2.267	**21.76** ± 3.482	**1.37** ± 0.499	**3.25** ± 0.654
Benzo(b)fluoranthene	**83.40** ± 70.45	**3.08** ± 1.685	**32.49** ± 18.71	**9.12** ± 1.759	**2.25** ± 1.123	**3.18** ± 0.957
Benzo(k)fluoranthene	**8.79** ± 3.128	**6.43** ± 2.099	**11.35** ± 5.667	**8.82** ± 2.412	**5.64** ± 2.043	**6.21** ± 2.574
Benzo(a)pyrene	**11.64** ± 2.204	**1.64** ± 0.361	**6.77** ± 1.884	**22.93** ± 6.301	**1.75** ± 0.675	**15.77** ± 3.831
Dibenz(a,h)anthracene	**444.33** ± 157.7	**2.50** ± 0.937	**553.50** ± 156.2	**79.81** ± 44.98	**1.93** ± 0.541	**235.08** ± 120.1
Benzo(g,h,i)perylene	**74.34** ± 56.55	**2.56** ± 0.711	**217.17** ± 47.52	**53.22** ± 9.807	**13.50** ± 10.511	**72.17** ± 33.75
Indeno(1,2,3-cd)pyrene	**1046.83** ± 476.2	**2.76** ± 1.356	**2073.35** ± 423.4	**309.61** ± 56.08	**3.12** ± 1.078	**751.93** ± 310.8
POPs						
∑HCHs	**0.81** ± 0.000	**6.89** ± 0.879	**0.69** ± 0.311	**2.73** ± 0.121	**19.9** ± 0.832	**0.65** ± 0.000
∑DDT	**4.66** ± 0.000	**19.0** ± 0.338	**1.44** ± 0.410	**21.5** ± 0.224	**7.69** ± 0.625	**1.70** ± 0.000
∑Endosulfan	**1.48** ± 0.000	**9.97** ± 1.213	**0.01** ± 0.041	**2.03** ± 0.135	**4.05** ± 0.570	**0.26** ± 0.000
∑Drines	**0.95** ± 0.000	**17.3** ± 1.738	**1.78** ± 0.497	**1.96** ± 0.099	**4.32** ± 0.590	**0.31** ± 0.000

The results from OneWay-ANOVA statistical analysis for PAHs and POPs analysed in the anemones and sediments collected from the three climate areas are described in Table 5. The Tukey results indicate the post hoc comparisons between climate areas (at both organism's tissues and sediments) and species. No data were obtained for Naphthalene, Acenaphthylene, Acenaphthene, Anthracene and Fluorene.The results showed significant differences between climate areas PAHs concentrations on organisms tissues for Naphthalene ($F_{(2,56)}$ 0.000), Anthracene ($F_{(2,56)}$ 0,034), Fluorene ($F_{(2,56)}$ 0,010), Pyrene ($F_{(2,56)}$ 0,009), Chrysene ($F_{(2,56)}$ 0.000), Benzo(a)pyrene ($F_{(2,56)}$ 0.000), Dibenz(a,h)anthracene ($F_{(2,56)}$ 0.000), Benzo(g,h,i)perylene ($F_{(2,56)}$ 0,001) and Indeno(1,2,3-cd)pyrene ($F_{(2,56)}$ 0.000) (Table 5). It were also observed significant differences between species PAHs concntrations for Naphthalene ($F_{(2, 56)}$ 0.001). Fluorene ($F_{(2,56)}$ 0,000), Fluoranthene ($F_{(2,56)}$ 0,016), Pyrene ($F_{(2,56)}$ 0,000), Chrysene ($F_{(2,56)}$ 0.000), Benzo(a)pyrene ($F_{(2,56)}$ 0.004), Dibenz(a,h)anthracene ($F_{(2,56)}$ 0.000), Benzo(g,h,i)perylene ($F_{(2,56)}$ 0,000) and Indeno(1,2,3-cd)pyrene ($F_{(2,56)}$ 0.000) (Table 5). Concerning sediments PAHs concentrations between climate areas it were observed significant differences for Fluoranthene ($F_{(2,56)}$ 0,039), Pyrene ($F_{(2,56)}$ 0,044), Chrysene ($F_{(2,56)}$ 0.042), Benzo(b)fluoranthene ($F_{(2,56)}$ 0.046), Benzo(g,h,i)perylene ($F_{(2,56)}$ 0,045) and Indeno(1,2,3-cd)pyrene ($F_{(2,56)}$ 0.044) (Table 5). The results showed significant differences between climate areas POPs concentrations on organisms tissues and species for all compounds (Table 4) and significant differences in sediments for ∑HCHs ($F_{(2,56)}$ 0,010) and ∑DDT ($F_{(2,56)}$ 0,004) (Table 5).

Table 5. Results from OneWay-ANOVA statistical analysis for PAHs and POPs (ug/g) analysed in the anemones and sediments collected from the three climate areas. The Tukey results indicate the post hoc comparisons between

climate areas (at both organisms tissues and sediments) and species; statistically different are indicated as bold ($p<0.05$). (*) No data were obtained for Naphthalene, Acenaphthylene, Acenaphthene, Anthracene and Fluorene.

PAHs	Organisms		Sediments
	Climate Zones	Species	Climate Zones
Naphthalene	$F_{(2,56)}$ **0.000**	$F_{(5,56)}$ **0.001**	(*)
Acenaphthylene	$F_{(2,56)}$ 0.106	$F_{(5,56)}$ 0.255	(*)
Acenaphthene	$F_{(2,56)}$ 0.067	$F_{(5,56)}$ 0.178	(*)
Anthracene	$F_{(2,56)}$ **0.034**	$F_{(5,56)}$ 0.064	(*)
Fluorene	$F_{(2,56)}$ **0.010**	$F_{(5,56)}$ **0.000**	(*)
Phenanthrene	$F_{(2,56)}$ 0.058	$F_{(5,56)}$ 0.229	$F_{(5,25)}$ 0.405
Fluoranthene	$F_{(2,56)}$ 0.065	$F_{(5,56)}$ **0.016**	$F_{(5,25)}$ **0.039**
Pyrene	$F_{(2,56)}$ **0.009**	$F_{(5,56)}$ **0.000**	$F_{(5,25)}$ **0.044**
Benzo(a)anthracene	$F_{(2,56)}$ 0.247	$F_{(5,56)}$ 0.733	$F_{(5,25)}$ 0.162
Chrysene	$F_{(2,56)}$ **0.000**	$F_{(5,56)}$ **0.000**	$F_{(5,25)}$ **0.042**
Benzo(b)fluoranthene	$F_{(2,56)}$ 0.294	$F_{(5,56)}$ 0.089	$F_{(5,25)}$ **0.046**
Benzo(k)fluoranthene	$F_{(2,56)}$ 0.865	$F_{(5,56)}$ 0.961	$F_{(5,25)}$ 0.777
Benzo(a)pyrene	$F_{(2,56)}$ **0.000**	$F_{(5,56)}$ **0.004**	$F_{(5,25)}$ 0.321
Dibenz(a,h)anthracene	$F_{(2,56)}$ **0.000**	$F_{(5,56)}$ **0.000**	$F_{(5,25)}$ 0.426
Benzo(g,h,i)perylene	$F_{(2,56)}$ **0.001**	$F_{(5,56)}$ **0.000**	$F_{(5,25)}$ **0.045**
Indeno(1,2,3-cd)pyrene	$F_{(2,56)}$ **0.000**	$F_{(5,56)}$ **0.000**	$F_{(5,25)}$ **0.044**
POPs			
∑HCHs	$F_{(2,18)}$ **0.000**	$F_{(5,18)}$ **0.000**	$F_{(2,11)}$ **0.010**
∑DDT	$F_{(2,18)}$ **0.000**	$F_{(5,18)}$ **0.000**	$F_{(2,11)}$ **0.004**
∑Endosulfan	$F_{(2,16)}$ **0.002**	$F_{(5,18)}$ **0.005**	$F_{(2,11)}$ 0.639
∑Drines	$F_{(2,16)}$ **0.016**	$F_{(5,18)}$ **0.008**	$F_{(2,11)}$ 0.513

The results for PAHs and POPs Bioconcentration Factors (BCFs) and OneWay-ANOVA statistical analysis are described in Table 6. The Tukey comparisons results indicate the post hoc comparisons between climate areas. No BCFs were obtained for Naphthalene, Acenaphthylene, Acenaphthene, Anthracene and Fluorene. Concerning PAHs the results showed, except for Phenanthrene, significant differences in Bioconcentration Factors for all compounds ($p<0.05$) (Table 5). In relation to POPs the results showed significant differences in Bioconcentration Factors only for ∑HCHs ($p<0.05$) (Table 6).

Table 6. PAHs and POPs Bioconcentration Factors (BCFs) and OneWay-ANOVA statistical analysis. The BCFs are indicated as mean ± SEM. The Tukey comparisons results indicate the post hoc comparisons between climate areas; statistically different are indicated as bold ($p<0.05$). No BCFs were obtained for Naphthalene, Acenaphthylene, Acenaphthene, Anthracene and Fluorene.

PAHs	Tropical	SubTropical	Temperate	1-way-Anova
Phenanthrene	**0.00** ± 0.000	**0.12** ± 0.123	**0.00** ± 0.000	$F_{(2,24)}$ 0.129
Fluoranthene	**0.00** ± 0.000	**2.18** ± 1.124	**0.00** ± 0.000	$F_{(2,24)}$ **0.014**
Pyrene	**9.70** ± 9.698	**0.47** ± 0.295	**1.17** ± 0.389	$F_{(2,24)}$ **0.036**
Benzo(a)anthracene	**2.79** ± 2.792	**8.08** ± 4.392	**1.97** ± 0.655	$F_{(2,24)}$ **0.038**
Chrysene	**1.02** ± 1.021	**3.03** ± 1.811	**0.00** ± 0.000	$F_{(2,24)}$ **0.038**
Benzo(b)fluoranthene	**2.63** ± 1.753	**49.54** ± 45.318	**3.21** ± 1.069	$F_{(2,24)}$ **0.040**
Benzo(k)fluoranthene	**3.84** ± 3.352	**0.74** ± 0.454	**3.95** ± 1.975	$F_{(2,24)}$ **0.048**
Benzo(a)pyrene	**6.85** ± 5.224	**10.10** ± 3.551	**2.58** ± 1.367	$F_{(2,24)}$ **0.012**
Dibenz(a,h)anthracene	**0.00** ± 0.000	**198.39** ± 114.412	**0.20** ± 0.066	$F_{(2,24)}$ **0.022**
Benzo(g,h,i)perylene	**0.00** ± 0.000	**77.48** ± 36.696	**4.93** ± 1.643	$F_{(2,24)}$ **0.008**
Indeno(1,2,3-cd)pyrene	**281.26** ± 183.624	**672.95** ± 399.964	**2.78** ± 0.959	$F_{(2,24)}$ **0.023**
POPs				
∑HCHs	**0.24** ± 0.026	**0.54** ± 0.183	**2.53** ± 0.278	$F_{(2,11)}$ **0.020**
∑DDT	**12.17** ± 0.950	**0.10** ± 0.067	**0.24** ± 0.086	$F_{(2,11)}$ 0.264
∑Endosulfan	**0.21** ± 0.037	**0.19** ± 0.124	**0.61** ± 0.164	$F_{(2,11)}$ 0.273
∑Drines	**0.13** ± 0.043	**0.07** ± 0.055	**0.80** ± 0.196	$F_{(2,11)}$ 0.115

PCO (Principal coordinate Analysis) and RDA (redundancy analysis)

The spatial and species PAHs and POPs variations in the organism's tissues bioconcentrations responses and comparisons by Principal Coordinate Analysis (PCO) using a Permutational Multivariate Analysis of Variance (PERMANOVA) are described in Figures 2-4. For PAHs and POPs in organisms tissues the first two axes accounted for 46.7% and 83.9 % of overall data variability, respectively (Figures 2A and 2B). Overall, for PAHs in organism's tissues results suggest that the first axis is associated to spatial variability between the three climate zones with a defined segregation of the temperate points from the Sub-Tropical and Tropical ones. The second axis is associated with the PAHs types, being evident the separation of the PAHs with 2 to 4 rings (Temperate areas) from 4-6 ring compounds (Sub-Tropical and Tropical areas) (Figure 2A). For POPs in organism's tissues, the spatial segregation was not so evident. Although in the second axis there is a clear separation of the temperate area ∑HCHs from all the other compounds (Figure 2B).

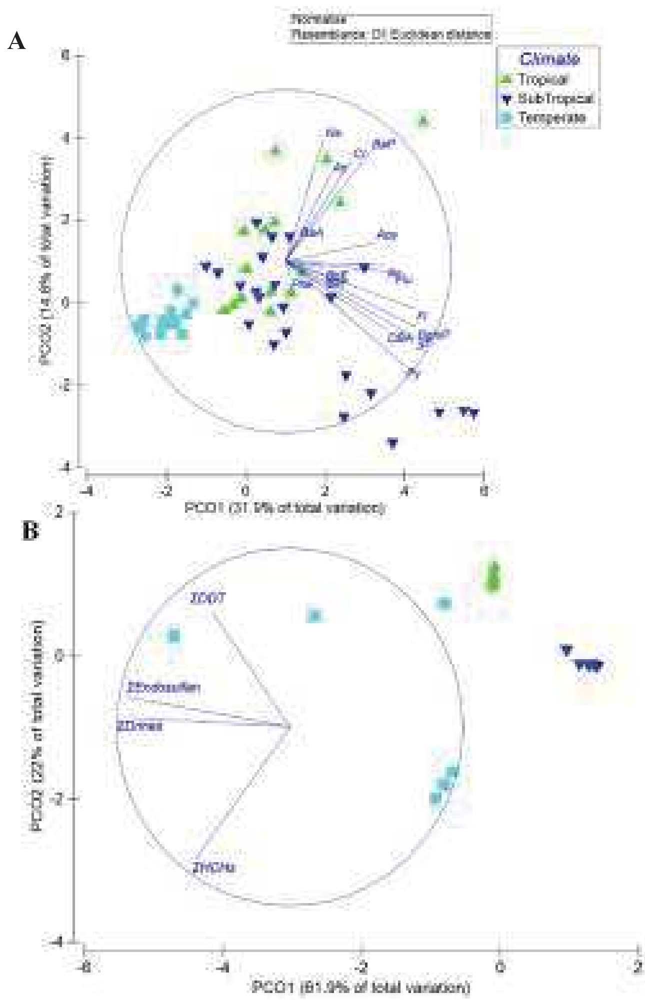

Figure 2. Principal Coordinate Analysis (PCO) scaling plot of the (A) PAHs and (B) POPs measured in the organisms tissues, collected in the three climate zones. Parameters vectors are also overlapping the scaling plot with a Pearson correlation of 0.2.

The spatial and species PAHs and POPs variations in the selected species,bioconcentrations responses and comparisons by Principal Coordinate Analysis (PCO) using a Permutational Multivariate Analysis of Variance (PERMANOVA) are described in Figures 3A and 3B. The first two axes accounted for 46.7% and 83.9 % of overall data variability, respectively (Figures 3A and 3B). Overall, results suggest that the first axis is associated to species variability between the three climate zones, with a clear separation of *A. equina* and *A. sulcata* (Temprate areas) from all the other species (Sub-Tropical and Tropical areas) (Figure 3A). The second axis is associated with the PAHs types, being evident the separation of the PAHs with 2 to 4 rings (Temperate areas) from 4-6 ring compounds (Sub-Tropical and Tropical areas) (Figure 3B). For POPs, it is also evident the separation of *A.equina* and A. sulcata from the other species (Figure 3A). In in the second axis there is a clear separation of *A. sulcata* and ∑HCHs from all the other species and compounds (Figure 3B).

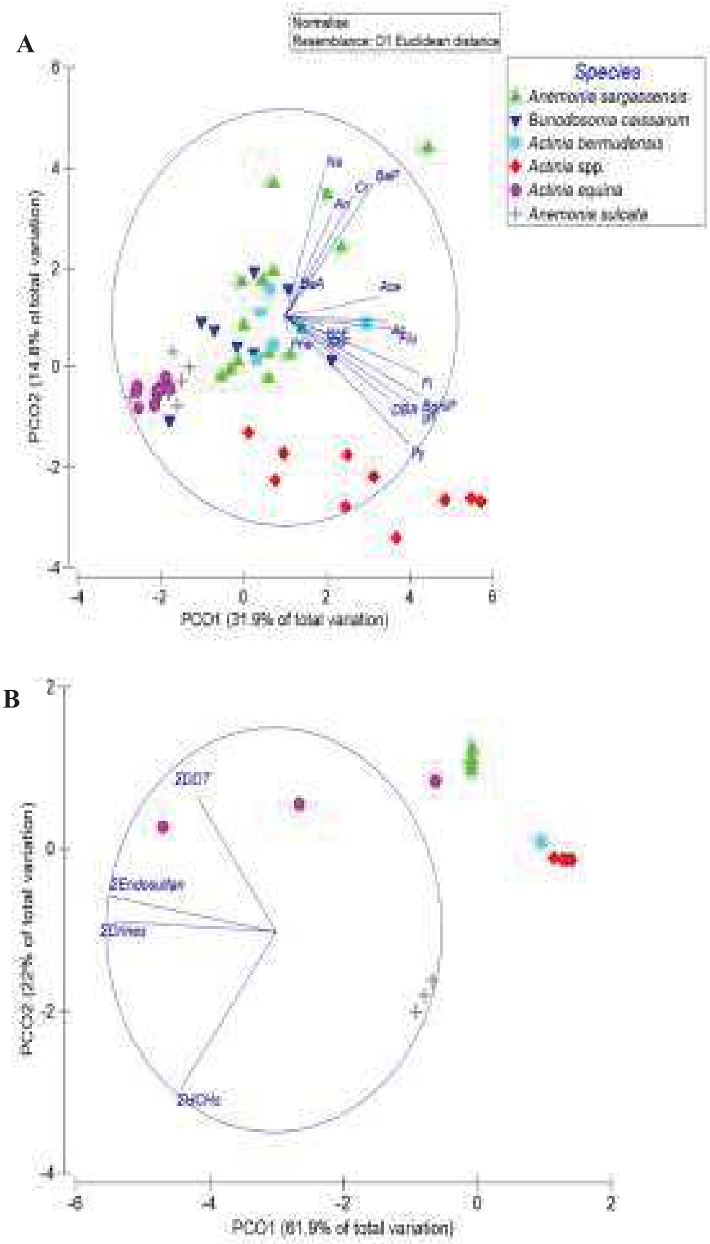

Figure 3. PCO scaling plot of the (A) PAHs and (B) POPs measured in the selected organisms, from the three climate areas. The selected Pearson correlation was 0.2.

The spatial PAHs and POPs variations in the sediments concentrations responses and comparisons by Principal Coordinate Analysis (PCO) using a Permutational Multivariate Analysis of Variance (PERMANOVA) are described in Figures 4A and 4B. The first two axes accounted for 78.4% and 80.2 % of overall data variability, respectively (Figures 4A and 4B). Overall, for PAHs in sediments results also suggest that the first axis is associated to spatial variability between the three climate zones with a defined segregation of the Sub-Tropical points from the Tropical and Temperate ones. The second axis is associated with the PAHs types, being evident the separation of the PAHs with 2 to 4 rings (Temperate and Tropical areas) from 4-6 ring compounds (Sub-Tropical areas) (Figure 4A). For POPs sediments, the first axis showed a clear segregation between ∑HCHs, ∑Drines and ∑Endosufan of Tropical and Sub-Tropical areas, from ∑DDT (Temperate and Sub-Tropical areas) (Figura 4A). In the second axis there is a clear separation of the ∑Drines and ∑DDT (Sub-tropical and Tropical areas) from all the other compounds (Figure 4B). The Figure 4C and 4D, showed a PCO scaling plot of the BCF, resulting from (C) PAHs and (D) POPs measured in organisms and sediments collected in the three climate zones. Parameters vectors are also overlapping the scaling plot with a Pearson correlation of 0.2.

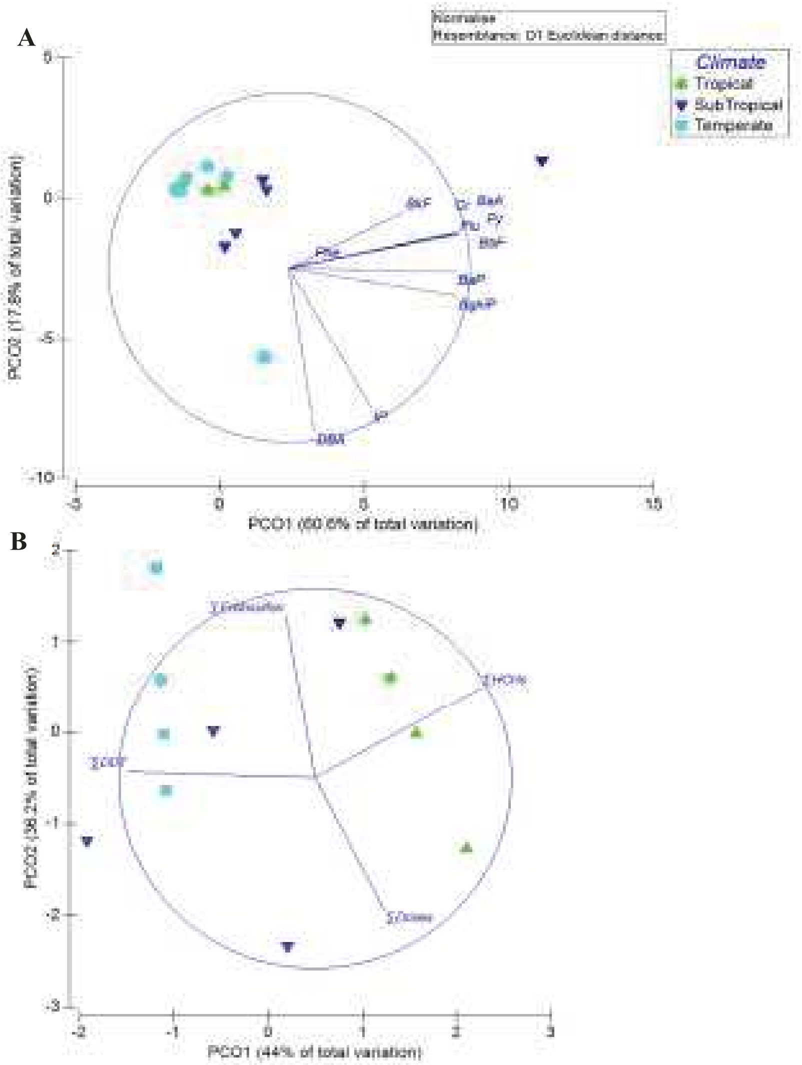

Figure 4. Principal Coordinate Analysis scaling plot of the (A) PAHs and (B) POPs measured in the sediments sampled from the three climate zones. Parameters vectors are also overlapping the scaling plot with a Pearson correlation of 0.2.

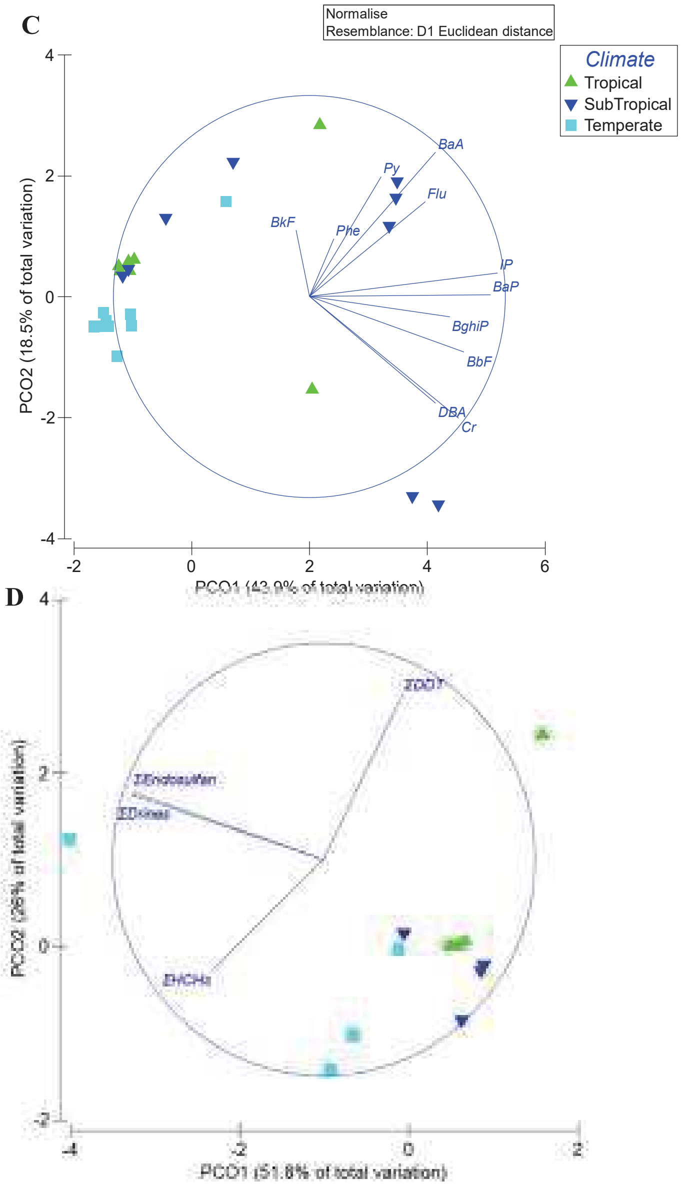

Figure 4. PCO scaling plot of the BCF, resulting from (C) PAHs and (D) POPs measured in organisms and sediments collected in the three climate zones. Parameters vectors are also overlapping the scaling plot with a Pearson correlation of 0.2.

The biological (contaminants analysed in tissues),environmental (concentrations in sediments) and selected anemones data, in the three selected climate areas analyzed by redundancy analysis (RDA) as environmental descriptors are shown in a triplot ordination diagram (Figures 5 and 6).

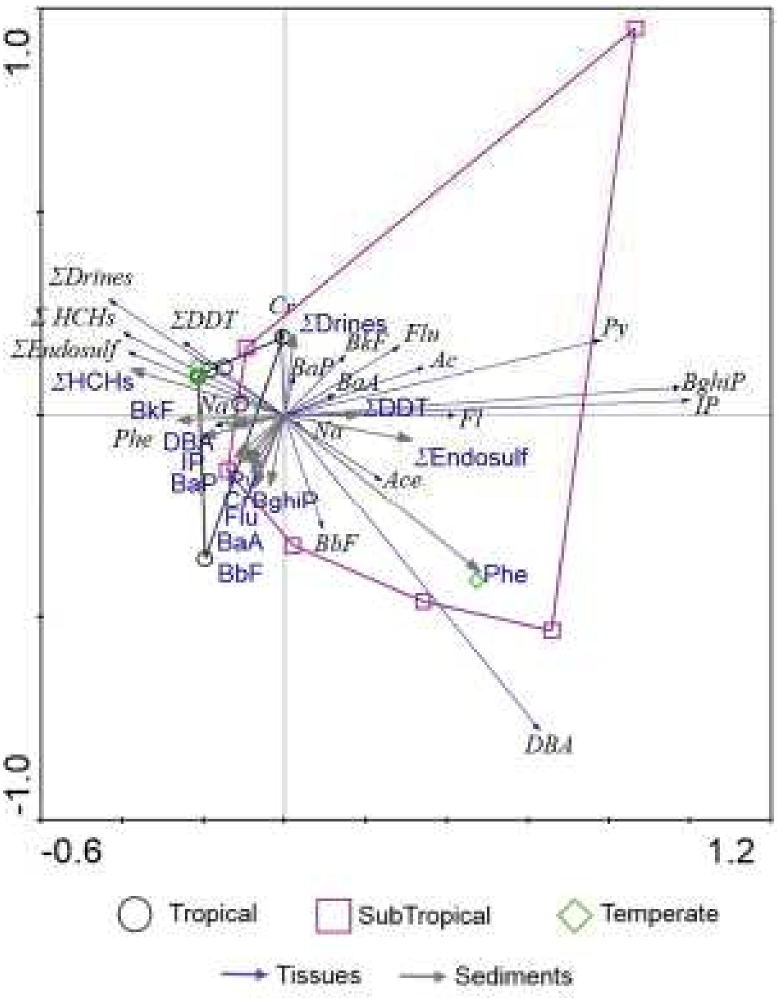

Figure 5. Redundancy analysis (RDA) ordination diagram with biological (contaminants analysed in tissues) and environmental (concentrations in sediments) data in the three selected climate areas. First axis is horizontal, second axis is vertical. Both axes explained 95.7% of total variability.

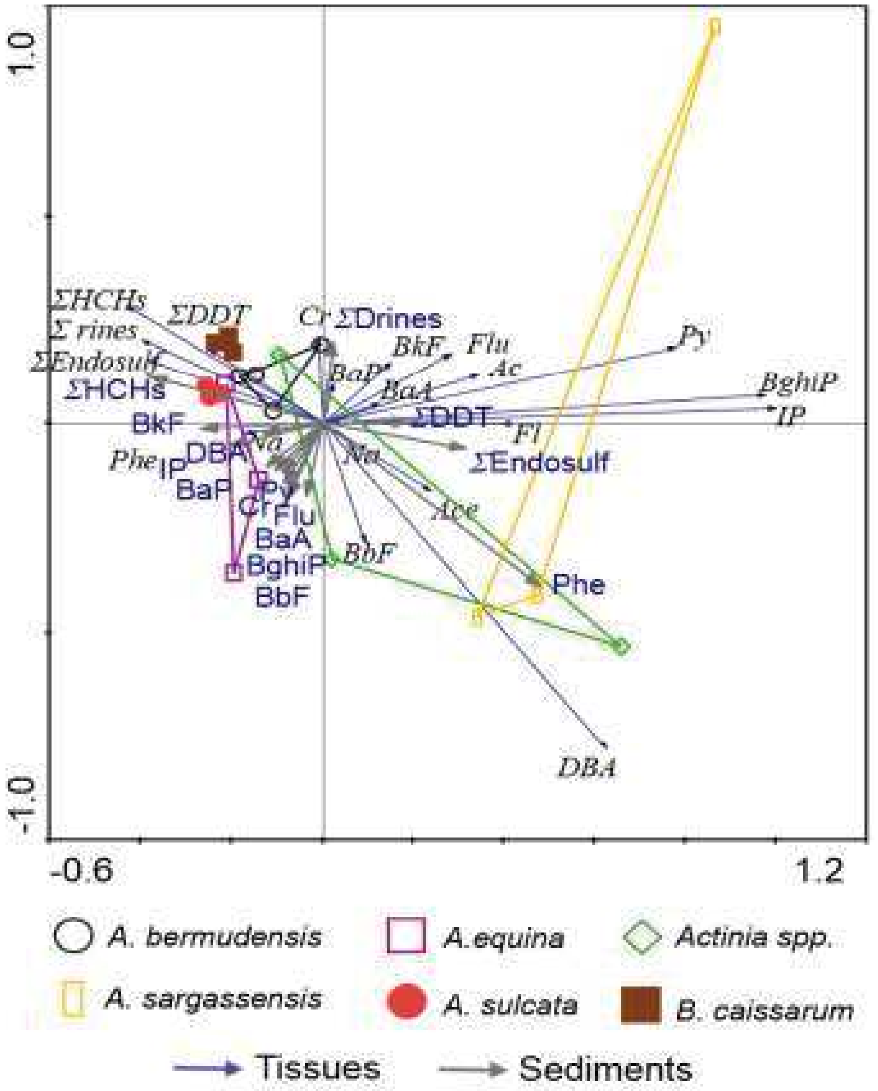

Figure 6. Redundancy analysis (RDA) ordination diagram with biological (contaminants analysed in tissues) and environmental (concentrations in sediments) data in selected anemones, collected from the three climate zones. First axis is horizontal, second axis is vertical. Both axes explained 89.4% of total variability.

Concerning the biological (contaminants analysed in tissues) and environmental (concentrations in sediments) the first two axes accounted for 95.7% of overall data canonical variability (Axis 1:77.2%; Axis 2:18.5%). While, it is evident that, in the first axis, there are an opposite pattern of PAHs and POPs bioaccumulation in tissues. It is also evident the separation of the PAHs with 2 to 4 rings from 4-6 ring compounds.In ths second axis, it is observed a clear segregation between the contaminants accumulation patterns between sediments and tissues, with the association of POPs and Temperate regions (Figure 5). Concerning the biological (contaminants analysed in selected species) and environmental (concentrations in sediments) the first two axes accounted for 89.4% of overall data canonical variability (Axis 1: 78.1%; Axis 2:18.5%). Overall, results suggest that there is a spatial separation associated to species variability between the three climate zones. It is observed that the species from the Tropical and Sub.Tropical areas, *A. Sargassensis* and *Actinia* spp, and the species from Temperate areas, *A. equina* and *A. sulcata*, are clearly separated from all the others. It is also evident the separation of *A. bermudensis* and *B. caissarum* from the other tropical species.

Discussion

Coastal areas are vulnerable to the accumulation of semivolatile organic compounds, such as PAHs, OCPs and PCBs from atmospheric inputs, fallouts from incomplete combustion, municipal effluents, and oil spillages and leaks of oil and refined oil products (Bartle *et al* 1991, Davis, 2003, Anyakora *et al* 2004). These chemical contaminants PAHs, CBs and chlorinated pesticides are known to be usually strongly associated with sediment, while being almost absent in the water phase. They mainly attach to the fine fraction while coarse particles present only few active spots.PAHs are found throughout the environment in the air, water and soil as complex mixtures (Jacobs 1994). Persistent Organic Pollutants (POPs) are chemical substances that persist in the environment and can bioaccumulate through the food web and pose a risk of causing adverse effects to human health and the environment. POPs include industrial chemicals like polychlorinated biphenyls (PCBs), combustion byproducts like dioxins, Polycyclic Aromatic Hydrocarbons (PAHs) and pesticides like Dichlorodiphenyl - trichloroethane (DDT) among others.

In this context, marine invertebrates are, therefore, important in the transfer of carbon and nutrients as well as OCs and PAHs to upper trophic level organisms. These invertebrates provide a link between phytoplankton and fish, seabirds and mammals in marine food webs. Sea anemones species are closely associated with the sediment and play an important role in marine ecosystems. Therefore, they have been shown to serve as valuablebio-indicators to monitor human activities, such as the disposal of dredged-material (Simonini et al., 2005). A knowledge of the trends and dynamics of OCs and PAHs between sediment and marine invertebrates is important for the understanding of trends and patterns of contaminants overall in marine ecosystems. For chemical analysis, species of interest must be limited in mobility, sufficiently present in the catch, important in the ecosystem and potential accumulators of pollutants. Clams, starfish, sea anemone, several crustaceans (brown shrimp, swimming crab and hermit crab) and fish (dragonet, goby and hooknose) are selected. In general, sea anemones could show the highest concentrations of persistent organic pollutants (POPs), due to filter feeding, the lack of sophisticated detoxification pathways and theirliving inside the sediment. Following the European directives guidelines of the Water Framework Directive and Marine Strategy Framework Directive, this study has provided data on the PAHs and POPs concentrations found on sediments, sea anemones tissues and in selected sea anemones species, in order to provide the evaluation of ecological and environmental status of the three different climatic coastal areas.

Chemical analysis (PCBs, DDTs, PAHs,) indicated the presence of different pollutants, and significant differences in sampling sites, both qualitative and quantitative, among the three climatic regions. Based on the accumulated levels of POPs and PAhs in sea anemones species, it can be concluded that there were significant differences in contamination between sites and climatic coastal areas sediments and also between organism's tissues. The results for PAHs sediments concentrations were much lower than levels reported for sea anemones tissues. The highest concentrations for PAHs sea anemones tissues concentrations were registered in Tropical and Sub-tropical climate coastal areas.The highest sediments concentrations were registered in temperate climate coastal areas. There are more than 100 PAHs, but the 16 USEPA priority compounds as proposed by the OSPAR guidelines and identified as priority pollutants by US Environmental Protection Agency (EPA) were present in sediments and bioacumulated by sea anemones in this study: Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo (a) anthracene, Chrysene, Benzo (b) fluoranthene, Benzo (k) fluoranthene, Benzo (a) pyrene, Indeno (1,2,3-cd) pyrene, Dibenzo (a, h) anthracene, and Benzo (g, h, i) pyrelene. The majority of the PAHs observed were 4 to 6-ring compounds. These PAHs display varying degrees of toxicity, but generally, the toxicity of PAHs increases with their molecular weight which potentially contribute too many of the modern day diseases including cancer, damage to the reproductive system, disrupted endocrine and immune systems, and neurobehavioral effects (Schirmer et al 1998; Hellou et al 2002). Transport to the marine environment may occur both via surface waters and atmosphere.in the water column most PAHs tend to absorb to particles and to be deposited to the underlying sediments. In the water column most PAHs do not dissolve but rather adsorb onto particles and are then deposited to the bottom sediment (Li et al, 1999; Bailey, 2001). The above activities could possible contribute to the high levels of polycyclic aromatic hydrocarbons (PAHs) in sediments and organisms tissues in the study sites, because the highest concentrations of PAHs are generally found around urban areas (Meador et al 1995). Although, in the temperate, tropical and subtropical coastal areas the levels of these compounds are in dependence of a number of cycles of condensation, deposition and re-evaporation, transferred from ambient air to other compartments of the environment by dry deposition and wet deposition, reaching a partitioning equilibrium according to temperature dependences and the vapor pressure of the chemicals (Semeena and Lammel, 2005). The obtained results highlight the anthropogenic pressure near coastlines and the chronic pollution, once the studed areas are impacted by harbour, industrial, agricultural and municipal activities that keep increasing with the ever growing population.

The most common OC compounds bioacumulated by sea anemones were the more water-soluble compounds, such as HCH isomers and lower chlorinated PCB congeners (Fisk et al., 2001b, 2003b; Hoekstra et al., 2002). Concerning DDT residues, results suggested a relatively recent use of the pesticide in the study areas. When DDT is introduced into the environment, it is converted to DDD andDDE, and despite that DDT has a halflife of 7 years, its metabolite DDE survives much longer and is the predominant form detected in fish and humans (Easton et al., 2002).
Endosulfan is a current use pesticide that is used globally and recently listed on the Stockholm Conven tion on POPs (Stockholm Convention, 2011). Endosulfans are used on a wide variety of crops and als

o for the control of disease vectors (Li and Macdonald, 2005). Since elimination occurs slowly, ongoing exposure may lead to an increase in the body burden over time. This reflects the smaller size, lower trophic level, and lack of biotransformation capacity generally found in invertebrates as compared to fish, mammals and birds. The results for POPs sea anemones tissues concentration were much lower than PAHs levels reported. The results for POPs sediments concentrations were generally much higher than levels reported for sea anemones tissues. The highest concentrations were registered in Temperate and Tropical climate coastal areas. The majority of the PAHs observed were ∑HCHs, ∑DDT, ∑Endosulfan and ∑Drines. Water is an important exposure route to OCs for sea anemones but recent evidence suggests that diet may also play a role (Hoekstra et al., 2002; Borga et al., 2004). The relative abundance of HCH probably reflects long-range atmospheric transport of the chemicals and geographic proximity to areas of recent application. The persistent organic pollutants (POPs), are very resistant to natural breakdown processes and therefore extremely stable and long-lived in the environment. These compounds are of concern as they are potentially carcinogenic, mutagenic, and have endocrine-disrupting impacts even onto mammals at the top of the food chain via bioaccumulation in the lipid fraction of biological tissues and biomagnifications in the wildlife and humans (Li, 1999; Bailey, 2001).

The selected sea anemones species showed, like other soft-bodied marine invertebrates so far studied (Howard and Brown, 1984; Brown, 1987; Scott, 1990; Guzman and Jimenez, 1992; Harland et al., 1990; Harland and Nganro, 1990; Mitchelmore et al., 2003), to be able to utilize organic substances dissolved in the sediments for their metabolism. Since sea anemones species differ in susceptibility towards disturbance, been adapted to different degrees of stress, the results of PAHs and POPs bioacumulation in this study determine differences of species assemblages among sites and climate areas. The biological (contaminants analysed in tissues), environmental (concentrations in sediments) and selected anemones data, in the three selected climate areas analyzed by Principal Component Analysis (PCO) redundancy analysis (RDA) as environmental descriptors showed an an opposite pattern of PAHs and POPs bioaccumulation in tissues. The results also showed a clear segregation between the contaminants accumulation patterns between sediments and tissues, with the association of POPs and Temperate regions. Concerning the biological (contaminants analysed in selected species) and environmental (concentrations in sediments), results also suggest that there is a spatial separation associated to species variability between the three climate zones. It is observed that the species from the Tropical and Sub-Tropical areas, *Actinia* spp and *A. sargassensis*, and the species from Temperate areas, *A. equina* and *A. sulcata*, are clearly separated from all the others. It is also evident the separation of *A. bermudensis* and *B. caissarum* from the other tropical species. The analysis of these results showed significant differences in PAHs Bioconcentration Factors for all compounds ($p<0.05$). and for ∑HCHs ($p<0.05$), being indicative of sea anemones as selective bioaccumulation indicators, possessing morphological and physiological adaptations to environmental forcing factors, which include eventual pollutants. This could be due to a combination of differential species susceptibility to biotransformation and variation in kinetics due to different physical– chemical properties (Denton and Burdon-Jones, 1986; Hanna and Muir, 1990).

Pollutants uptake occurs problably via ingestion of contaminated food, and accumulation and depuration processes, can occur during sea anemone life cycle depending on the diet, water conditions and individual susceptibility for environmental contaminants. These organisms may have the ability to accumulate large quantities of PAHs and POPs compounds from the environment, and also plays an important role in the storage, redistribution and detoxification (Lobban & Harrison, 1997; Harland et al., 1990; Hurd, 2000). These results confirm the importance of filter feeding organisms, such as sea anemones, potential for pollutants bioaccumulation, coupled with the slow rate of excretion and metabolism, leads to biomagnification, from marine sediments to the food chain (Mason et al., 2006).

Overall, the obtained results indicate significant differences in biological responses and pollutant loads among the selected sea anemones species and along the three selected climatic coastal areas and reinforce the importance of selecting different sentinel species from different habitats for the assessment of pollution impact in the marine environment. The analysis of PAHs and POPs in sediments and in organisms tissues gave an indication of recent exposure to The study provides further support for the use of sea anemones in assessing the health of coastal areas, being suitable as sentinel species for future pollution biomonitoring studies.

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CHAPTER 4
Chemical Stressors Effects on Natural Populations

Chapter 4. Article 3 to submission on Chemosphere (Status *submitted*).

Response of oxidative stress biomarkers in sea anemones from three climatic coastal environments

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ABSTRACT

The sea anemones has a wide distribution in the Atlantic and coastal areas and it may be found in both contaminated and pristine areas. It may then be assumed that the selected species *Anemonia sargassensis, Anemonia sulcata, Actinia bermudensis, Actinia equina, Bunodosoma cangicum* and *Bunodosoma caissarum* could be a valid tool for evaluation studies of environmental contamination. The goal of this study was to detect the spatial and interspecific activity variations of GST, GR, CAT, LPO and SOD as environmental biomarkers, on natural populations of sea anemones, under environmental stress and sources of pollution in three countries with different climatic scenarios. It were observed significant differences between all enzymatic activity vs climatic area, GR, $F=4.975$, $p< 0.001$), CAT, $F=4.282$, $p< 0.001$), GST, 7.569, $p< 0.001$), LPO, $F=75.300$, $p< 0.001$) and SOD, $F=5.226$, $p< 0.001$) (Permanova, Pseudo $F= 18.28$, $p<0.01$). The multiple comparisons among climate area x enzymatic activities also showed interspecific differences between all enzymes activities (PERMANOVA (Multiple Comparisons) analysis $p< 0.001$). In general the organisms from contaminated stations exhibited significantly lower mean enzymes activity levels than in reference stations (ANOSIM, Global R: 0.55, $p< 0.01$). It was revealed an interspecific variation in oxidative stress enzymes responses. Both *A. equina* and *A. sulcata* contributed to a better differentiation between reference and contaminated areas. The studied sea anemones revealed a number of properties that make them useful sentinels for environmental monitoring and can provide a measure of environmental pollution.

Keywords: Sea anemones; Bioindicators; Oxidative Stress Biomarkers; Coastal Environments; Climatic Zones.

1. INTRODUCTION

At the geographic scale marine organisms are exposed to a wide variety of environmental factors on varying temporal and spatial scales, from polar to tropical, in order to maintain homeostasis, growth and to reproduction (Cascade et al. 2011; Lesser, 2006; Birchenough et al. 2010 and Suggett et al. 2012). They are also subject to multiple stress factors as a result of anthropogenic activities that overload the environment with substances of both industrial and/or natural origins promoting antagonistic or combined effects and adjustment at different levels of biological organization (Lee and Mitchell-Olds, 2006 and Halpern et al. 2008). Pollutants are also known to cause oxidative stress resulting from contaminant uncontrolled reactive oxygen species (ROS) production or an imbalanced state between antioxidant defenses and ROS production is seen occurring in living organisms (Fukada et al. 2011). The combined effects of chemical and forced natural factors such as climatic shifts may cause significant ecological risks and adverse health effects in wildlife populations, namely on diversity, fecundity and reproductive competence (Moreira et al. 2005). Organisms have developed an evolutionary capacity to tolerate environmental variability and changes by means of several mechanisms, such as behavioral adaptations, morphological alterations (intra and interspecific variation), regulation of reproduction, and cellular responses (Parmesan, 2006). The physiological performance which reflects their evolutional adaptation to local environment (Dong et al. 2011; Somero, 2012), could provide a direct link between thermal physiology and animals ecology, which elucidates the role of aerobic metabolism in the thermal tolerance of many aquatic animals across latitudes

(Somero, 2012). These expressions precede population-level changes and are useful indicators if linked to specific physiological or ecological events (Dong et al. 2011; Lesser, 2006; Abele et al. 2011 and Lushchak, 2011). Consequently, baseline studies of natural and anthropogenic impacts on natural populations are necessary to understand the risks posed to marine habitats. Biochemical biomarkers, including antioxidant enzymes and evidence of oxidative damage to biomolecules, are powerful tools for detecting the exposure and biological effects of pollutants, allowing early detection of environmental problems and may reveal biological effects induced by pollutants under the influence of different climatic marine scenarios. Aquatic organisms exhibit a variety of changes in enzymatic antioxidant defenses after exposure to pollutants with oxidative potential (Regoli et al., 2002a,b). Prominent among these antioxidant defense system are the superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione-S-transferases (GSTs). In addition, Lipid peroxidation (LPO) levels have been used successfully as a measure of xenobiotic-induced oxidative stress (Lucesoli and Fraga, 1995; Reinheckel et al., 1998).

The majority of toxicological data of the last decade for most chemical contaminants originates from temperate species and locations and there has been an increase importance in studies comparing the effects of relevant contaminants also for tropical marine species. However, neither have extensive reports been published nor has a comparison of enzymatic levels with other areas in the Atlantic coast been carried out. Among macrofaunal organisms used in biomonitoring, both communities and individual species of bivalves, echinoderms (Beiras et al. 2003), sponges (Stupak et al. 2003), anemones (Gadelha et al. 2010), crustaceans (Crawford et al. 2003) and fishes (Nabekura et al. 2002) have usually been used as environment bioindicators or biomonitors (Hanna and Muir, 1990) for this purpose. Studies have been reported on the use of soft corals or coral tissues for monitoring purposes (Hanna and Muir, 1990) and as indicators of morphological and physiological adaptations to environmental forcing factors, which include eventual pollutants. Sea anemones are among the most abundant representatives and cosmopolitan species of coastal benthic communities (ca 6000 species) inhabit virtually all marine environments. They are abundant, ecologically diverse and can be found at different regions, included in contaminated areas (Rodríguez et al. 2007). These cnidarians are present on rocks and other hard substrates in shallow coastal water and like other soft-bodied marine invertebrates (symbiotic relationship) so far studied are able to utilize organic substances dissolved in the sea for their metabolism. The selected sea anemones: *Actinia equina, Anemonia sulcata* (Portuguese coast), *Anemonia sargassensis*, *Actinia bermudensis*, *Bunodosoma caissarum* e *Bunodosoma cangicum* (Brasilian and Mexican coast) are marine organisms that are directly exposed, at different latitudes and ecoclimatic regions, to extreme environmental conditions, continually contaminated exposure, exposed to wastes, untreated sewage inflow, land and river runoff, atmospheric fallout from heavy traffic and various small-scale industries scattered in coastal areas, which may cause physiology alterations.

In order to answer these hypotheses it was developed studies of the enzymatic biomarkers responses on the selected sea anemones species. The objectives of this work were (1) to study the enzymatic responses of oxidative stress biomarkers in anemones from three countries (different climatic conditions); (2) to compare enzymatic activities of six anemones from similar ecological niches, (3) to determinate the different responses of biomarkers according to polluted and non-polluted location.

2. MATERIALS AND METHODS

2.1 Sample sites

To undertake an integrated monitoring strategy seven sampling sites were selected in the north, south and western Atlantic coast under the influence of different climatic environments and types of contamination (Figure. 1). The study locations include reference and contaminated areas under anthropogenic, industrial and harbor influence combining exposed and sheltered habitats. Table 1 shows the locations and geographic coordinates of each the sampling sites.

Table 1. Sampling sites with locations and coordinates information of each climatic scenario.

Species	Sampling Sites	Location	Coordinates	Climatic Environment
Actinia equina, Anemonia sulcata	Vila Praia de Âncora (VPA)	Northwest, Portugal	41°49'13.54"N 8°52'26.05"W	Temperate
Actinia equina, Anemonia sulcata	Viana do Castelo Praia Norte (PN)	Northwest, Portugal	41°41'41.61"N8°51'6.4 3"W	Temperate
Anemonia sargassensis, Bunodossoma cangicum	Itamaracá (ITA)	Recife, Brazil	7º47'S 34º50'W	Tropical
Anemonia sargassenssis	Olinda (OLI)	Recife, Brazil	7°58'27.21"S 34°49'54.46"W	Tropical
Actinia bermudensis	Punta Xen (PXE)	Campeche, Mexico	43° 2'56.80"W 19°20'8.91"N	Sub-Tropical
Actinia bermudensis	Siho Playa (SHP)	Campeche, Mexico	90°43'37.92"W19°33'2 4.10"N	Sub-Tropical
Actinia bermudensis, Bunodosoma caissarum	Itaipu (NIT)	Rio de Janeiro, Brazil	22°58'18.10"S 43° 2'56.80"W	Sub-Tropical

2.1.1. Tropical environment

The study was undertaken in Itamaracá, a tropical tidal estuarine system (Figure 1), located in Pernambuco in northeastern Brazil, 35 km north of Recife. It is a natural and under low antropogenic influence environmental area (situated in APA Environment protection area) [85 and 86]. The other study area in the tropical environment was Olinda – Northest coast Brazil the Casa Caiada - Rio Doce beach complex, a 4.5 km- long sandy coastline located at the northern end of Olinda City (Pernambuco, NE Brazil) (Figure. 1). The characteristics of this stressed coastal area were determined, first by those of the surrounding coastal sea and second, by the strong influence of urban activities (Pereira et al. 2003; DHN, 1974). In southern tropical environmental Itamaracá (ITA) (Pernambuco, Brazil) was selected as reference station and Olinda (OLI) a displayed contaminated area (Pernambuco, Brazil), described as suffering the influence of anthropogenic and urban and/or industrial contamination pressures (Cerqueira and Pio, 1999).

2.1.2. Sub-tropical environment

The study area extends in the Itaipú bay (Niterói, Rio de Janeiro State- southern coast Brazil) near the Imbuí Point on the west to the Itaipú Point on the east, containing the Piratininga, Camboinhas and Itaipú beaches, and its seaward limit corresponds to the aligned islands of Pai, Mãe, and Menina (Figure. 1). Despite the enormous growth of population along the shoreline in Niterói, the water depth varies from a minimum of about 3-4 m (just seaward of the average breaking wave zone) to a maximum of 28 m, all of these depths pertaining to the shoreface environment (DHN, 1974; ECP, 1999). The other studied area was the coastline Gulf of México, eastern part of Campeche (México). The Punta Xen (PXE) is the reference site, situated on protected area, were marine turtles comes to nidification every year and is a permanently monitoring site. The other place is Siho Playa (SHP) is a degraded environment, with visible urban distribution. Both places receive a strongly influence by the marine current comes from USA Atlantic coast and carry out the Petroleum platform influence near of these place. In the southern sub-tropical environment three locals were selected, Punta Xen (PXE), Siho Playa (SHP) (Campeche, Mexico) and Itaipu (NIT) (Rio de Janeiro, Brazil) (Figure 1, Table 1). The NIT point were located the sampling stations with agricultural, harbor and anthropogenic influence this has been described as a heavy metals polluted site (Monterroso, 2005).

2.1.3. Temperate environment

For the temperate environment, the study was carried on the Portuguese coast that is divided in three main regions: North, Centre and South. This division was made considering that Portugal is the southern geographical limit for many boreal species and the northern or western limit of subtropical and Mediterranean species (Saldanha, 1974). Two sites were selected in northwest temperate environment (Portugal), Vila Praia de Âncora, reference station (VPA), Praia Norte (PN). Concerning the sampling stations located along the NW Portuguese coast: VPA – Vila Praia de Âncora and PN-Praia Norte. VPA is located near small fishery villages and far from big population aggregates and potential sources of contamination (Agricultural, Urban and Harbour). Several studies performed in this coast indicated that this site is relatively undisturbed by anthropogenic pressures (Moreira, 2004). PN is located in the vicinity of important industrial facilities, namely an oil refinery and a harbor supporting intensive vessel traffic; thus, they are chronically exposed to petroleum-derived hydrocarbons, including PAHs and heavy metals (Serra, 1998; Hargitt, 1908).

2.2. Target species and sampling

Six target species were selected according their ecological importance, distribution and abundance. For the tropical environment it were selected *Anemonia sargassensis* (Hargitt, 1908) and *Bunodosoma cangicum* that are the most abundant sea anemones of Brazilian coast (Zamponi et al. 1998), with large distribution in medio and infralitoral habitats (Gomes and Mayal, 1997; Amaral et al. 2002). For the temperate environment it was selected *Actinia equina* (Cornelius et al. 1995) and *Anemonia sulcata* (Cornelius et al. 1995) that are the most representative species found on rocky shores of the European coast and as far as the coast of West Africa (Its range extends throughout the Atlantic coasts of Europe (Gadelha et al. 2010), North Africa and into the Mediterranean and South Africa (Stephenson, 1935). For the subtropical environment were selected two representative species, *Actinia bermudensis* and *Bunodosoma caissarum,* common on rocks just below the low tide line, that presents large distributions occurred since the West Indies, Bermuda and northern Florida and South to Brazil (Ruppert and Fox, 1988). Twenty sea anemones were collected (May to September 2012), at low tide, in the inter-tidal zone of the seven sampling sites (Figure 1.). The organisms collected were 1-3 cm in length. Samples were kept on ice during transport. From these, 20 animals of each species where used for biomarkers. Organisms were placed in thermally insulated boxes, previously filled with local water, and transported to the laboratory within 1–2 h of sampling. Selected tissues were used for biomarker determinations.

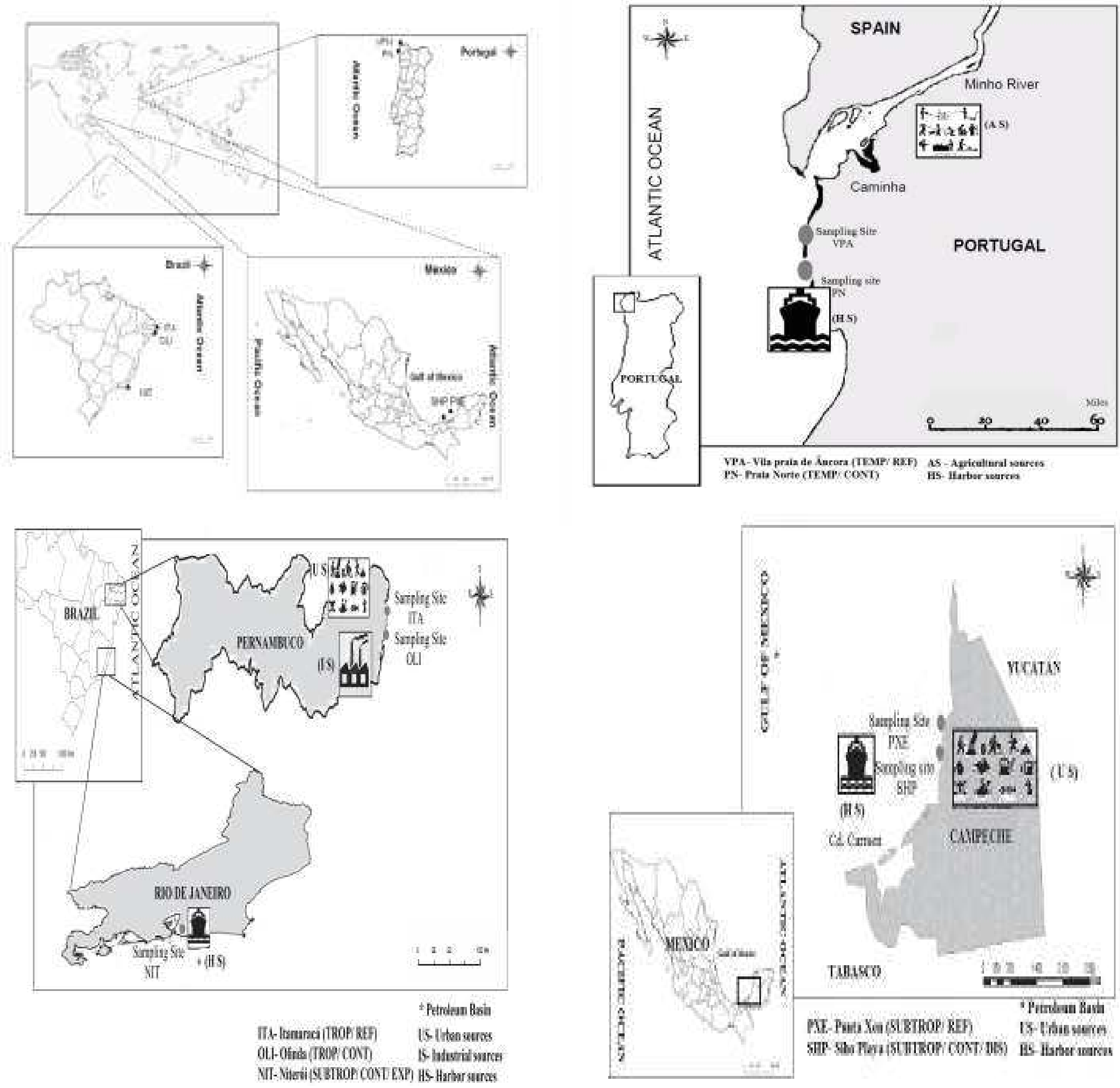


Figure. 1. Location of the sampling sites in the three climatic environments. Northwest Portuguese coast. Vila Praia de Âncora (VPA-reference; Viana do Castelo Praia Norte (PN)-urban and industrial effluents. Brazilian coast, Itamaracá (ITA)- reference, Olinda (OLI)I-urban and Niterói (NIT) industrial effluents. Mexican coast, Punta Xen (PXE)-reference, Siho Playa (SHP) urban and industrial effluents.

2.3. Enzymatic assays

Endoderm anemone tissues were isolated, homogenized (Ystral homogenizer, Ballrechten-Dottingen, Germany) in appropriate buffers, and centrifuged (SIGMA 3 K 30) at 4 °C. All methodologies applied follow standard procedures with few modifications.

LPO levels were measured by quantification of thiobarbituric acid reactive substances (TBARS) and expressed as nmol TBARS/g tissue (Ohkawa et al., 1979). GST was assessed according to Habig et al. (1974), with some modifications of the original protocol. The activities of GR (Cribb et al. 1989), SOD (Suzuki et al. 2000) were measured in microplates. CAT activity was measured according to the method of Aebi (1984). All the enzymatic determinations were carried out in a microplate Multiskan Spectrum Thermo spectrophotometer. Enzymatic activities were determined at 25 °C and expressed as activity per mg of protein (second protein determination performed after the enzymatic analysis, as indicated below). One unit (U) of SOD activity was defined as the amount of enzyme required to inhibit the rate of reduction of cytochrome c by 50%. For CAT, 1 U was defined as 1 μmol min^{-1}. After enzymatic analysis, the amount of protein in each sample was determined again and this value was used to express enzymatic activities. All the protein determinations were done by the Bradford method (Bradford, 1976) adapted to microplate.

2.4. Statistics

The mean values of the species enzymatic activities from the three environmental scenarios and each population are presented graphically in boxplots diagrams, using IBM SPSS Statistic, version 20 (IBM Corporation, 2011).

Data analysis was performed Permutational Multivariate Analysis of Variance PERMANOVA (Anderson, 2001) to test the effects of the factors in data structure. The data treatment was based on a crossed factorial design, consisting of the three factors 'enzyme type' (five levels, fixed), 'climatic location' (seven levels, fixed), and 'congeneric species' (seven levels, fixed). Logarithmic transformations were applied to reduce the influence of overwhelmingly abundant species. Non-metric multidimensional scaling (MDS) was used to produce two-dimensional ordination plots. One-way ANOSIM was used to test the null hypothesis of no significant differences between: (1) 'enzyme type'; (2) climatic location' and (3) "species'. ANOSIM test produces a statistic (R-statistic) that lies in the range (-1; 1). Values of RZ1 are obtained only when all replicates within groups are more similar to each other than any replicates from different groups (Clarke and Warwick, 2001).

Where group differences in community structure were found ($\alpha<0.05$ in PERMANOVA tests), we used another exploratory method to identify those species most responsible for the difference. SIMPER allowed us to calculate the total similarity within and dissimilarity between enzymes activities. For any two groups, SIMPER (similarity percentages) calculates the percent contribution each species makes to the total between group dissimilarity (Clarke and Warwick, 2001). The Bray–Curtis coefficient was used to construct the similarity matrix from the square root transformed data. For the tests, 9999 permutations were used. A significance level (p) of <0.05 was considered. The Bray–Curtis coefficient was used to construct the similarity matrix from the square root transformed data. For the tests, 9999 permutations were used. A significance level (p) of <0.05 was considered. The spatial and species biomarkers responses variations were analysed by Principal Coordinate Analysis (PCO) and compared using a Permutational Multivariate Analysis of Variance (PERMANOVA).The multidimensional scaling (MDS) ordination method was used to visualize the spatial and species biomarker responses. The biological and environmental data were also analysed by redundancy analysis (RDA), using enzymatic biomarkers as biological descriptors and water parameters as environmental descriptors. Multivariate PERMANOVA, PCO and MDS tests were performed using PRIMER with PERMANOVA+ software (PRIMER v6 & PERMANOVA+ v1, PRIMER-E Ltd.) The RDA analysis was performed using the software CANOCO 4.5 for Windows (Biometris,TheNetherlands).

3. RESULTS

3.1. Enzymatic activity vs climate area.

Enzymatic activities are shown in Figure. 2 and detailed results of PERMANOVAs separated by climate areas reported in Table 2. The enzymatic responses of oxidative stress biomarkers showed important average activity differences between the climatic scenarios. In tropical coastal areas the mean activities were for SOD (11173 ± 9045.06), CAT (92.2±44.84), GST (115.03±59.28), LPO (12.0 ± 4.40) and GR (8.0 ±3.89). For subtropical coastal areas the average mean activities were for SOD (67895.81 ± 14930.94), CAT (48.4±34.50), GST (76.30 ±41.72), LPO (5.4 ± 2.16) and GR (8.1 ± 2.21). In the temperate coastal areas the mean activities were for SOD (2493.04 ± 1806.36), CAT (31.4±18.48), GST (114.82±38.78), LPO (12.0 ± 4.43,0 ± 0.330) and GR (10 ± 1.93) (Figure 2). It were observed significant differences between all enzymatic activity vs climatic area, GR, F=4.975, $p < 0.001$), CAT, F=4.282, $p < 0.001$), GST, 7.569, $p < 0.001$), LPO, F=75.300, $p < 0.001$) and SOD, F=5.226, $p < 0.001$) (Permanova, Pseudo F= 18.28, $p<0.01$). SIMPER differentiates the enzyme activities contribution as being influential in the three coastal areas. For tropical areas the main contributions were due to GST, GR and LPO activities, with contributions of 36.84%, 28.96% and 25.94%, respectively. For sub-tropical areas the main contribution was due to SOD and GR activities, with 69.18% and 13.81%, respectively. For temperate areas the main contributions were due to GST and GR activities with 66.71% and 23.64%, respectively. The multiple comparisons among climate area x enzymatic activities also showed interspecific differences between all enzymes activities (PERMANOVA (Multiple Comparisons) analysis $p < 0.001$) (Table 2).

3.2. Enzymatic activity vs species vs climatic area

The specific mean enzymatic responses also showed considerable variations among anemone species and climatic area, being, in some cases, from two to four folds higher than the lowest mean activity (Figure 2). In tropical coastal areas the mean activities of *A. sargassensis* and *B. canjicum* were for SOD (11173 ± 9045.06), CAT (92.2±44.84), GST (115.03±59.28), LPO (12.0 ± 4.40) and GR (8.0 ±3.89). For subtropical coastal areas the average mean activities of *A. bermudensis* and *B. caissarum* were for SOD (67895.81 ± 14930.94), CAT (48.4±34.50), GST (76.30 ±41.72), LPO (5.4 ± 2.16) and GR (8.1 ± 2.21). In the temperate coastal areas the mean activities of *A. equina* and *A. sulcata* were for SOD (2493.04 ± 1806.36), CAT (31.4±18.48), GST (114.82±38.78), LPO (12.0 ± 4.43,0 ± 0.330) and GR (10 ± 1.93) (Figure 2).The GR activities minimum values were registered in *B. canjicum,* of 1.54434 ± 0.376013, and maximum values were registered in *A. sargassensis*, of 15.55948 ± 2.968243, observed in tropical coastal areas. The CAT activities minimum values were registered in *A. equina*, of 7.73 ± 3.39739, in temperate coastal areas, and maximum registered in *A. sargassensis*, of 137.8998 ± 11.17055, in tropical coastal areas (Figure 2). The GST activities minimum values were registered in *B. caissarum*, of 11.14 ± 5.43199, in sub-tropical coastal areas and maximum values registered in *A. sargassensis*, of 333.6812 ±

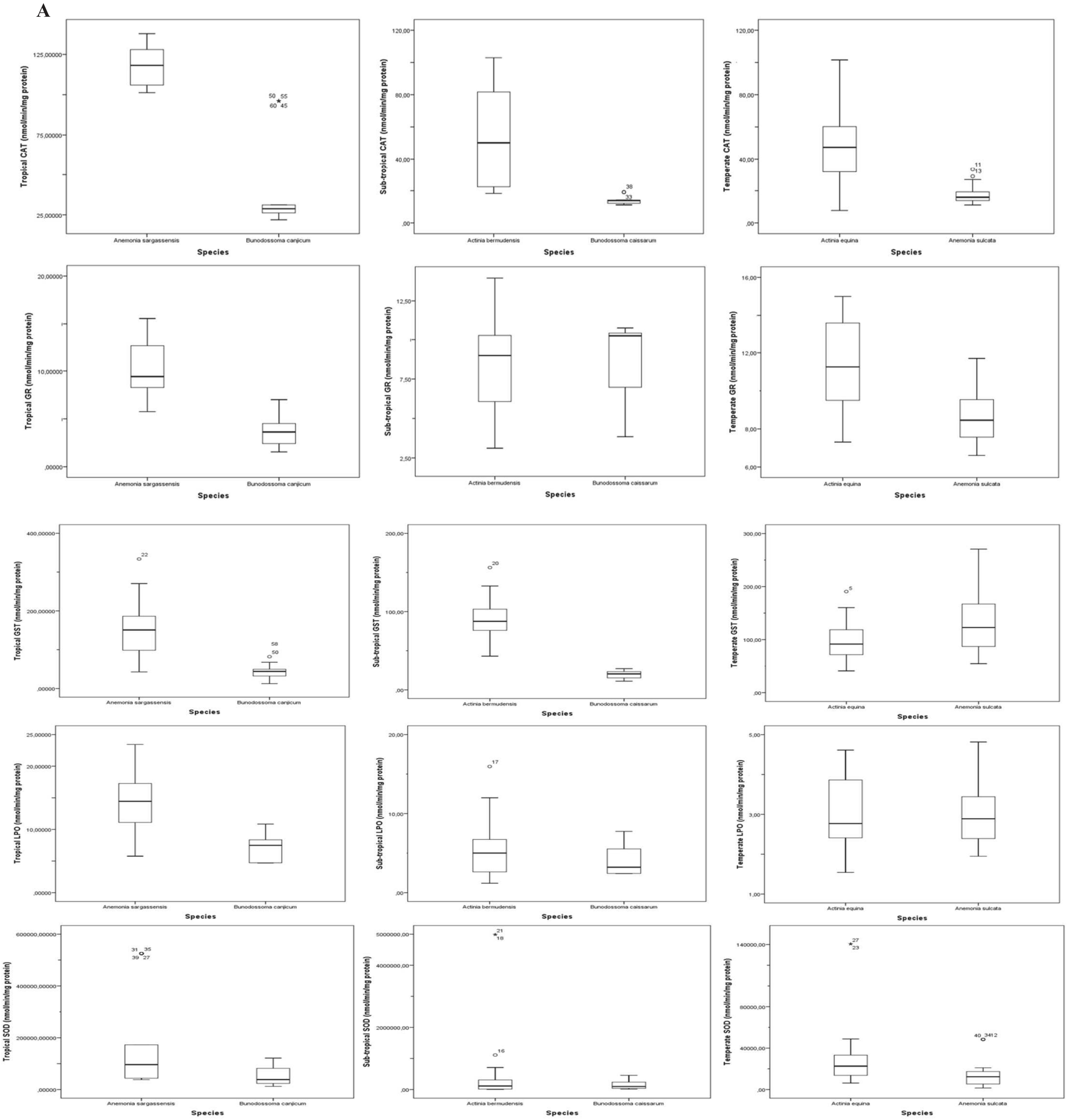
61.10863, in tropical coastal areas. The LPO activities minimum values were registered in *Actinia equina* registered in temperate coastal areas. The SOD activities minimum values were registered in *A. sulcata*, of 1530.81 ± 3377.837, in temperate coastal areas and maximum values in *A. bermudensis*, of 4986847 ± 172830.7, in sub-tropical coastal areas

Table 2. Results of PERMANOVA (Multiple Comparisons) analysis (Climatic coastal areas x anemone enzymes activities) of the effects of Climatic coastal areas and anemone species on multivariate enzymes activities ($p<0.01$).

Multiple Comparisons			
Dependent Variable	**(I) Climate**	**(J) Climate**	**Sig.**
GR	Tropical	Temperate	.009
	Sub-tropical	Temperate	.038
	Temperate	Tropical	.009
CAT	Tropical	Sub-tropical	.000
		Temperate	.000
GST	Tropical	Sub-tropical	.001
	Sub-tropical	Temperate	.005
LPO	Tropical	Sub-tropical	.000
		Temperate	.000
	Sub-tropical	Tropical	.000
	Temperate	Tropical	.000
SOD	Tropical	Sub-tropical	.029
		Temperate	.008

*. The mean difference is significant at the 0.05 level.

A



Tropical CAT (nmol/min/mg protein)
Sub-tropical CAT (nmol/min/mg protein)
Temperate CAT (nmol/min/mg protein)
Tropical GR (nmol/min/mg protein)
Sub-tropical GR (nmol/min/mg protein)
Temperate GR (nmol/min/mg protein)
Tropical GST (nmol/min/mg protein)
Sub-tropical GST (nmol/min/mg protein)
Temperate GST (nmol/min/mg protein)
Tropical LPO (nmol/min/mg protein)
Sub-tropical LPO (nmol/min/mg protein)
Temperate LPO (nmol/min/mg protein)
Tropical SOD (nmol/min/mg protein)
Sub-tropical SOD (nmol/min/mg protein)
Temperate SOD (nmol/min/mg protein)
Species
Anemonia sargassensis
Bunodossoma canjicum
Actinia bermudensis
Bunodossoma caissarum
Actinia equina
Anemonia sulcata

B

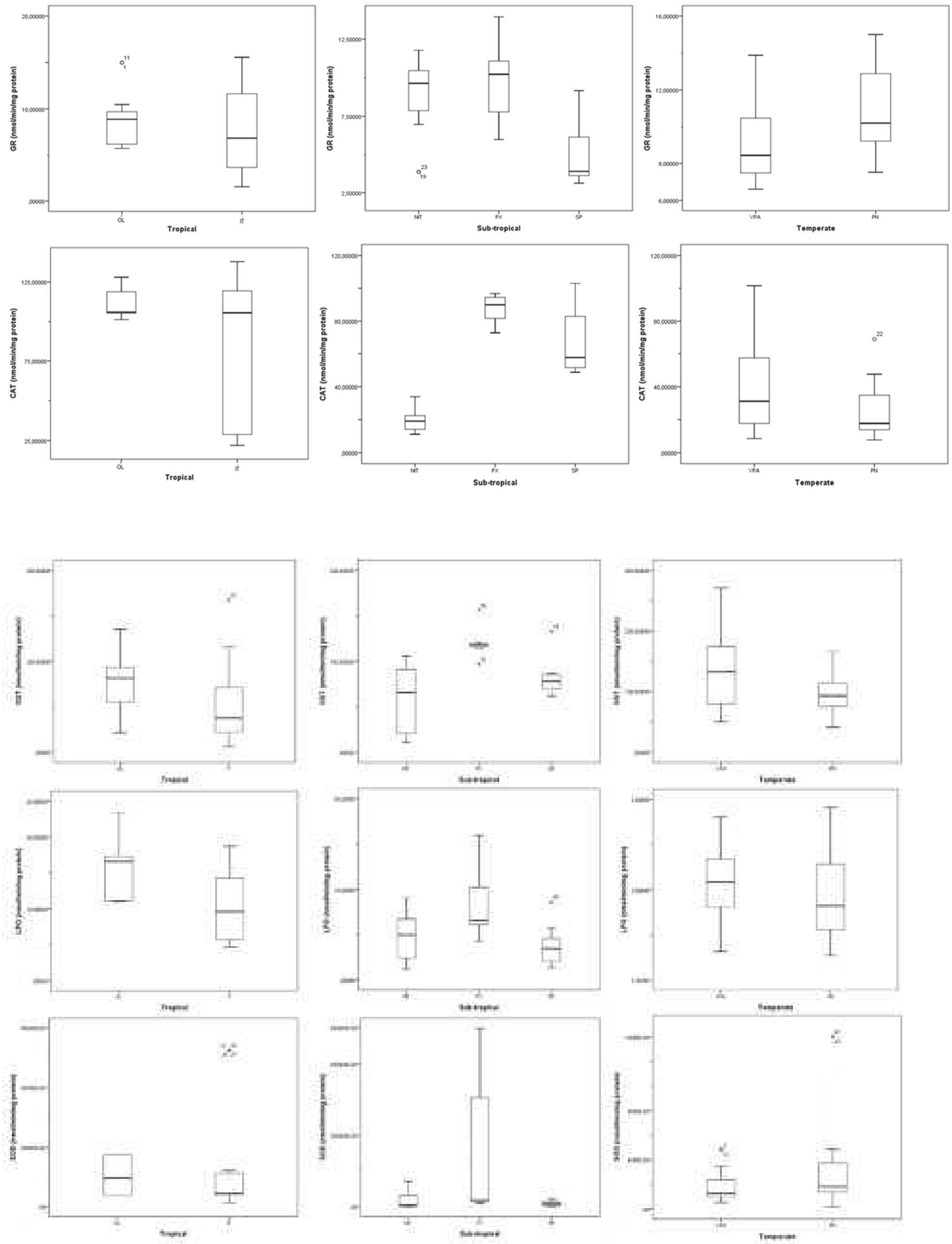


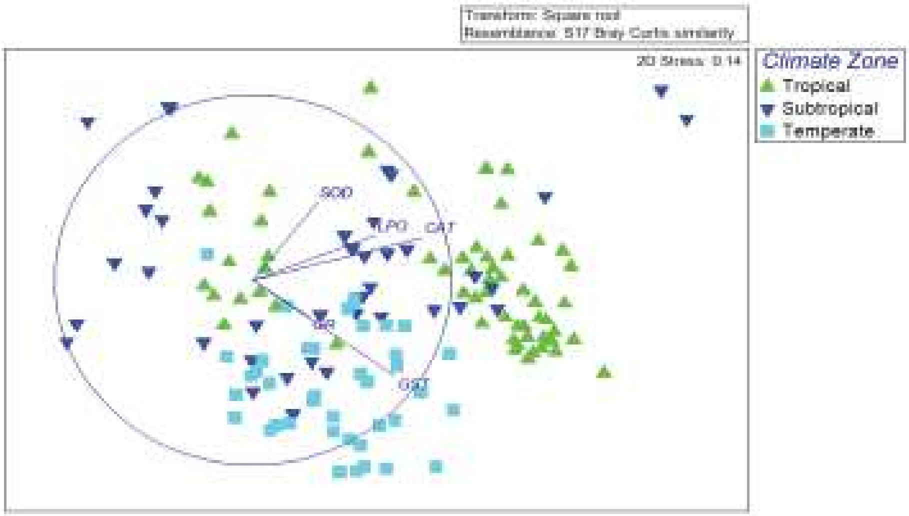
Figure 2. "Box-plot" representation of the stress biomarkers enzymes responses, of the six anemones species (A), from the three climate coastal areas (B). Boundaries of the boxes closest to, and furthest from zero indicate the 25th and 75th percentiles, respectively. The thin and thick lines within the Box mark the median and average,

respectively. Bars above and below the box indicate the 90th and 10th percentiles, respectively. Outliers are represented as black dots. (Permanova, p<0.01) (*), ANOSIM (One way analysis of similarities, Global R: 0.495, p< 0.01) (*).

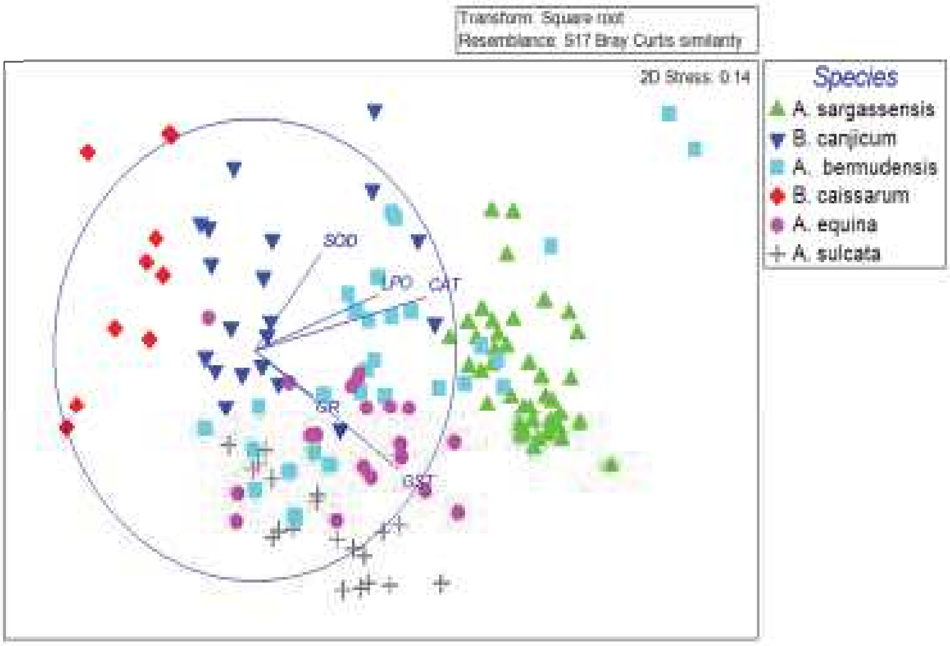
PERMANOVA revealed that the enzymatic activities of the anemones species showed significantly differences and was influenced by the climatic shift (Pseudo F=18.28, p<0.001) (Figure 3; Table 2).

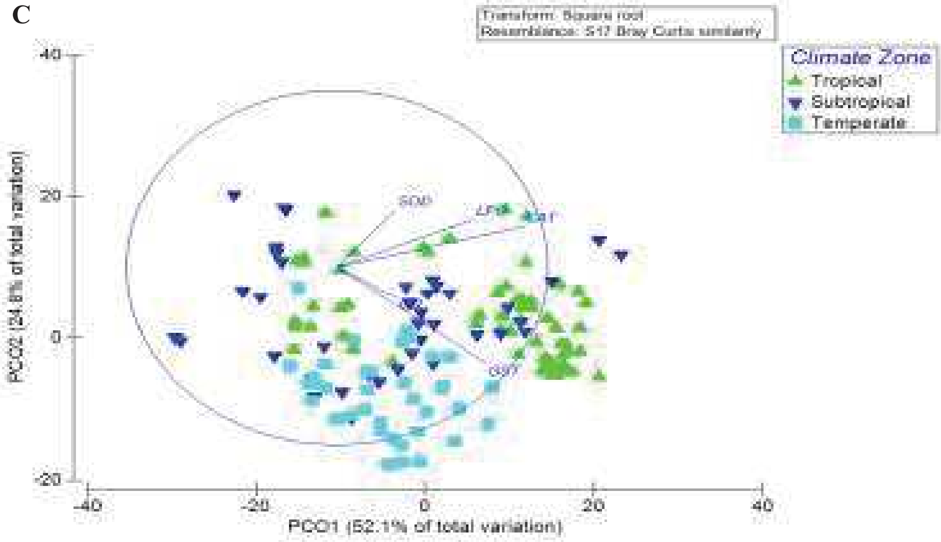
3.3. MDS (Multiple Dimensional Scaling), PCO (Principal coordinate Analysis) and RDA (redundancy analysis).
The multidimensional scaling (MDS) ordination method to visualize the spatial and species biomarker responses are described in Figures 3A and 3B. The spatial and species biomarkers responses variations and comparisons by Principal Coordinate Analysis (PCO) using a Permutational Multivariate Analysis of Variance (PERMANOVA) are described in Figures 3C and 3D. Points on the MDS with greatest separation represent parameters with greatest differences in enzymes activities and anemones species structure. The patterns in sea anemones and enzymes structure, for each climatic coastal area, and the similarities to each other are represented based upon dissimilarities. Data points represent the structure of enzymes activities and anemones species from replicate exclusion areas in each climate coastal area. The biological and environmental data analyzed by redundancy analysis (RDA), using enzymatic biomarkers as biological descriptors and water parameters as environmental descriptors and described in (Figure 4). While it is evident that there are substantial differences in enzymatic activities and also in anemones species among climate coastal areas. Overall results suggest SOD as the main discriminant biomarker, followed by CAT and LPO. The selected biomarker responses, observed in organisms captured along the three climate zones, indicate that *A. sargassensis*, *A. bermudensis* and *A. equina* revealed to be the most sensitive organisms in the tropical, subtropical and temperate areas, respectively. The results of RDA are shown in a triplot ordination diagram (Figure 4). The first two axes of the accounted for 91.9%, of overall canonical variability of the data. The first axis was associated to spatial variability and strongly characterized by the higher levels of superoxide dismutase, with higher levels on organisms collected in the subtropical areas. This axis is also characterized by the association between higher temperature, conductivity and salinity values with the higher LPO and CAT activities in the tropical and subtropical locations. The pH and GR values represented the lowest discriminative environmental and biological descriptors, respectively. The second axis was characterized by the opposition between temperatures and DO values. The DO levels were inversely correlated with LPO and CAT activities.

A


Transform: Square root
Resemblance: S17 Bray Curtis similarity
2D Stress: 0.14
Climate Zone
Tropical
Subtropical
Temperate
SOD
LPO
CAT
GR
GST

B


Transform: Square root
Resemblance: S17 Bray Curtis similarity
2D Stress: 0.14
Species
A. sargassensis
B. canjicum
A. bermudensis
B. caissarum
A. equina
A. sulcata
SOD
LPO
CAT
GR
GST



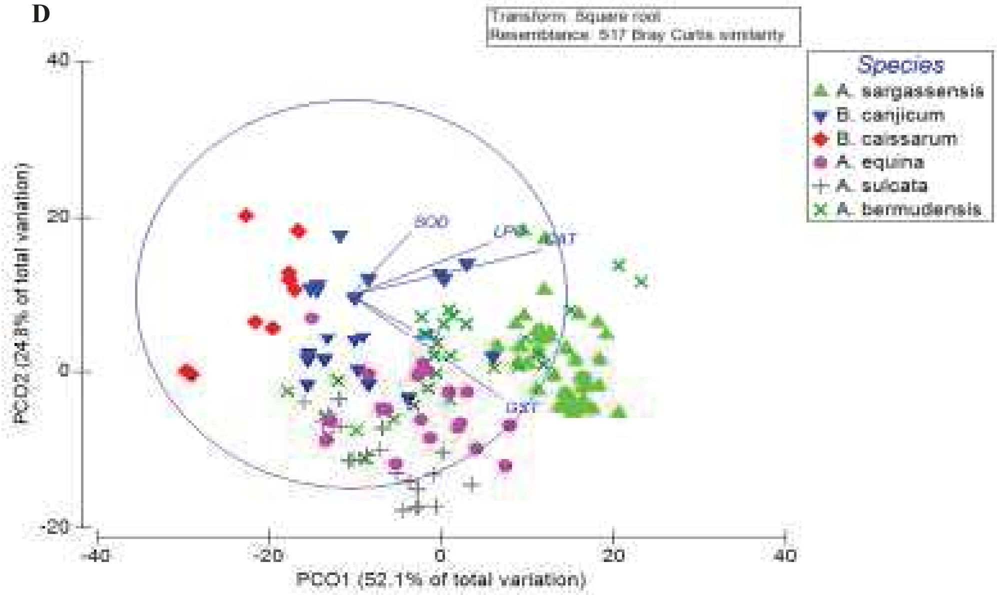


Figure 3. 2D MDS ordination plot for the biomarkers response arranged by studied climate areas (**A**) and selected species (**B**). Principal Coordinate Analysis (PCO) scaling plot of the three climate zones enzymatic responses (**C**) and the six selected species with biomarker responses (**D**). Biomarkers vectors are also overlapping the scaling plot with a Pearson correlation of 0.2.

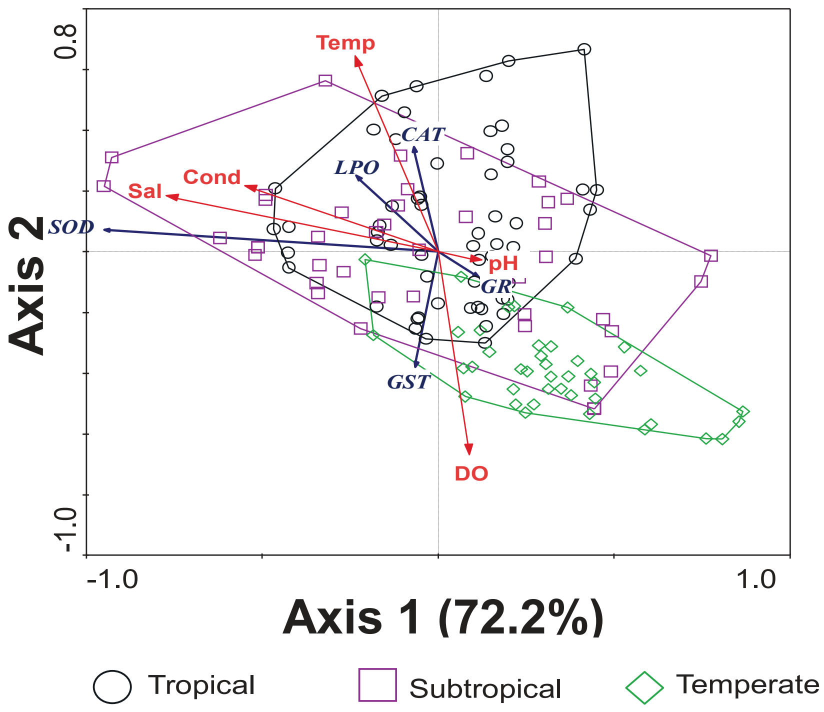


Figure 4. Redundancy analysis (RDA) ordination diagram with biological and environmental data in the three selected climate areas. The enzymatic responses were select as biological descriptors. First axis is horizontal, second axis is vertical. Environmental data: Temp – temperature, Sal- salinity, DO – dissolved oxygen and Cond – conductivity. Both axes explained 91.9% of total variability.

It were also observed significant differences between all enzymatic activity vs species, (PERMANOVA, GR, F=22,100, p< 0.001), CAT, F=97,911, p< 0.001), GST, F=26,212, p< 0.001), LPO, F=57,672, p< 0.001) and SOD, F=2,614, p< 0.001) (PERMANOVA (Multiple Comparisons), p<0.01, Table 2).

ANOSIM (Analysis of similarities) testing between all three coastal climatic areas identified a significant difference in the anemones species according to climate area. (One way, Global R: 0.495, p< 0.01) (Table 3).

Table 3. Analysis of similarity (one-way ANOSIM) for the anemone species (being pooled as replicates) for each climatic coastal areas (stations being pooled as replicates). Values obtained by ANOSIM are Global R statistic: (Global R): 0.495; Significance level of sample statistic: 0.01%

GROUPS	STATISTIC	LEVEL %
S1 *Anemonia sargassensis*, S2 *Bunodossoma canjicum*	0.867	0.01
S1 *Anemonia sargassensis*, S3 *Actinia bermudensis*	0.631	0.01
S1 *Anemonia sargassensis*, S4 *Bunodossoma caissarum*	0.921	0.01
S1 *Anemonia sargassensis*, S 6 *Actinia equina*	0.604	0.01
S1 *Anemonia sargassensis*, S5 *Anemonia sulcata*	0.862	0.01
S1 *Anemonia sargassensis*, S6 Actinia equina	0.802	0.01
S2 *Bunodossoma canjicum*, S3 *Actinia bermudensis*	0.282	0.01
S2 *Bunodossoma canjicum*, S4 *Bunodossoma caissarum*	0.575	0.01

S2 *Bunodossoma canjicum*, S 6 *Actinia equina**	0.882	0.01
S2 *Bunodossoma canjicum*, S5 *Anemonia sulcata*	0.839	0.01
S2 *Bunodossoma canjicum*, S6 *Actinia equina***	0.922	0.01
S4 *Bunodossoma caissarum*, S6 *Actinia equina**	0.614	0.01
S4 *Bunodossoma caissarum*, S5 *Anemonia sulcata*	0.615	0.01
S4 *Bunodossoma caissarum*, S6 *Actinia equina***	0.615	0.01
S6 *Actinia equina*, S5 *Anemonia sulcata*	0.413	0.02
S5 *Anemonia sulcata*, S6 *Actinia equina*	0.399	0.02

*control; **contaminated

When examined in finer detail the enzymes activities *vs* anemone species patterns (specifically, among all combinations of the fixed factors), using a two-way crossed PERMANOVA type-III design, the method cumulating samples provided greater replication and more degrees of freedom for each factor, it was observed that all interaction with each climatic coastal area vs anemone species were also significant (ANOSIM, Global R statistic: (Global R): 0.55, $P<0.01$, Table 4).

The differences in the multiple comparison pairwise results ranged from large differences in the biomarkers activities between species from tropical and subtropical coastal areas and less differences between the tropical and temperate areas (Global R: 0.55, $p< 0.01$) (Table 4).

Table 4. Results of the two-way analysis of similarity (ANOSIM) comparison for the for the anemone species among (being pooled as replicates) for each climatic coastal areas (stations being pooled as replicates). Values obtained by ANOSIM are Global R statistic:(Global R): 0.55; Significance level of sample statistic: 0.01%

GROUPS	STATISTIC	LEVEL %
S1 *Anemonia sargassensis*. S2 *Bunodossoma canjicum*	0.867	0.01
S6 *Actinia equina**. S5 *Anemonia sulcata**	0.413	0.06
S5 *Anemonia sulcata***. S6 *Actinia equina***	0.399	0.01

***control **contaminated**

SIMPER (similarity percentages) calculates the percent contribution of biomarkers activities, in each species, that makes to the total between group dissimilarity. SIMPER identifies a small subset of species that are more consistently present or more abundant in one group than another, thus helping to reveal the major contributors to each group's biotic identity and simplifying the interpretation of community patterns (Table 5).

Table 5. SIMPER (one-way (A) and two way (B) "between") results indicating the top stress oxidative biomarkers activities contributing up to 90% dissimilarity between anemone species.

(A) One-way "*between*"

Species	Enzymes	Av.Sq.Dist	Contrib%
A. sargassensis & *B. canjicum*	GR	4.54	30.15
	GST	4.22	27.99
	CAT	3.68	24.43
	LPO	2.54	16.82
A. sargassensis & *A. bermudensis*	SOD	4.32	29.04
	LPO	3.71	24.96
	CAT	2.86	19.21
	GST	2.14	14.42
	GR	1.84	12.38
B. canjicum & *A. bermudensis*	SOD	4.45	46.86
	GR	2.62	27.55
	CAT	0.918	9.67
	GST	0.862	9.08
A. sargassensis & *B. caissarum*	CAT	5.88	33.43
	GST	5.81	33.02
	LPO	4.14	23.56
B. canjicum & *B. caissarum*	GR	2.87	61.07
	CAT	0.807	17.17
	LPO	0.591	12.57
A. bermudensis & *B. caissarum*	SOD	4.27	47.58
	GST	1.6	17.78
	GR	1.37	15.23
	CAT	1.21	13.44
A. sargassensis & *A. equina*	LPO	44.41	
	CAT	22.57	44.41

	GST	22.16	22.57
	GR	9.86	22.16
B.canjicum & A. equina	GR	61.24	9.86
	GST	17.82	61.24
			17.82
	CAT	11.43	
A. bermudensis & A. equina	SOD	58.51	11.43
	GR	18.3	58.51
	CAT	9.06	18.3
	GST	7.61	9.06
B. caissarum & A. equina	GST	42.86	7.61
	CAT	25.8	42.86
	GR	24.59	25.8
A. sargassensis & A.sulcata	CAT	40.49	24.59
	LPO	36.31	40.49
	GST	14.26	36.31
B. canjicum & A. Sulcata	GST	43.38	14.26
	GR	35.3	43.38
	LPO	10.92	35.3
	CAT	10.27	10.92
A. bermudensis & A. sulcata	SOD	53.33	10.27
	GST	16.92	53.33
	CAT	12.69	16.92
	GR	10.52	12.69
B. caissarum & A. sulcata	GST	79.06	10.52
	GR	14.13	79.06
A. equina & A.	GST	47.75	14.13

		CAT	31.66	47.75
		GR	19.18	31.66
A.sargassensis	*& A. equina*	LPO	39.61	19.18
		CAT	30.7	39.61
		GST	15.37	30.7
		GR	13.59	15.37
B. cangicum	*& A.equina*	GR	72.95	13.59
		GST	12.36	72.95
		LPO	8.77	12.36
A. bermudensis	*& A.equina*	SOD	48.6	8.77
		GR	31.43	48.6
		CAT	8.19	31.43
		LPO	6.79	8.19
B.caissarum	*& A. Equina*	GR	46.96	6.79
		GST	39.49	46.96
		CAT	6.53	39.49
A. equina	*& A. equina*	GR	44.35	6.53
		GST	27.33	44.35
		CAT	25.39	27.33
A. Sulcata	*& A.equina*	GR	52.63	25.39
		GST	38.06	52.63

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(B) Two-way "*between*"

A. sargassensis	*& B. canjicum*	GR	4.54	30.15
		GST	4.22	27.99
		CAT	3.68	24.43
		LPO	2.54	16.82
A. Bermudensis	*B. caissaru...*	SOD	4.27	47.58
		GST	1.6	17.78

		GR	1.37	15.23
		CAT	1.21	13.44
A. equina	*A. Sulcata*	GST	1.64	47.75
		CAT	1.08	31.66
		GR	0.657	19.18
A. equina	*A. equina*	GR	1.18	44.35
		GST	0.727	27.33
		CAT	0.675	25.39
A. Sulcata	*A. equina*	GR	1.94	52.63
		GST	1.4	38.06

* control

**contaminated

The SIMPER (similarity percentages) between species from the three climatic coastal areas, showed that for *A. sargassensis* (Tropical coastal area) *vs B. canjicum (*Tropical coastal area), the main contributions to dissimilarity were the GR (30.15%), GST (27.99%), CAT (24.43%) and LPO (16.82%) enzymes activities. For *A. sargassensis* (Tropical area) *vs A. bermudensis* (sub-Tropical area), the main contributions to dissimilarity were the SOD (29.04%), LPO (24.96%), CAT (19.21%, GST (14.42 %) and GR (12.38%) enzymes activities. For *B. canjicum (*Tropical coastal area) *vs A. bermudensis* (sub-Tropical area), the main contributions to dissimilarity were the SOD (46.86%) and GR (27.55%), enzymes activities. For *B. canjicum (*Tropical coastal area) *vs B. caissarum* (sub-Tropical area), the main contributions to dissimilarity were the GR (61.97%), CAT (17.17%) and LPO (12.57%) enzymes activities. For *A. bermudensis* (sub-Tropical area) *vs B. caissarum* (sub-Tropical area), the main contributions to dissimilarity were the SOD (47.58%), GST (17.78%), GR (15.23%) and CAT (15.44%) enzymes activities. For *A. sargassensis* (Tropical area) *vs A. equina* (Temperate area), the main contributions to dissimilarity were the LPO (44.41%), CAT (22.57%) and GST (22.16%) enzymes activities. For *B. canjicum (*Tropical coastal area) *vs A. equina* (Temperate area), the main contributions to dissimilarity were the GR (61.24%), GST (17.82%) and CAT (11.43%) enzymes activities, For *A. bermudensis* (sub-Tropical area) *vs A. equina* (Temperate area), the main contributions to dissimilarity were the SOD (58.51%) and GR (18.3%) enzymes activities. For *B. canjicum (*Tropical coastal area) *vs A. equina* (Temperate area), the main contributions to dissimilarity were the GST (42.86%), CAT (25.8%) and GR (24.58%) enzymes activities. For *A. sargassensis* (Tropical area) *vs A. sulcata* (Temperate area), the main contributions to dissimilarity were the CAT (40.49%), LPO (36.31%) and GST (14.26%) enzymes activities. For *B. canjicum (*Tropical coastal area) *vs A. sulcata* (Temperate area), the main contributions to dissimilarity were the GST (43.38%), GR (35.3%), LPO (10.92%) and CAT (10.27%) enzymes activities. For *A. bermudensis* (sub-Tropical area) *vs A. sulcata* (Temperate area), the main contributions to dissimilarity were the SOD (53.33%), GST (16.92%), CAT (12.69%) and GR (10.52%)

enzymes activities. For *B. canjicum (*Tropical coastal area) *vs A. sulcata* (Temperate area), the main contributions to dissimilarity were the GST (79.06%) enzymes activities. For *A. equina* (Temperate area) *vs A. sulcata* (Temperate area) the main contributions to dissimilarity were the GST (47.75%) and CAT (31.66%) enzymes activities (Table 6).

Table 6. SIMPER (one-way and two way "Within") results indicating the top stress oxidative biomarkers activities contributing up to 90% dissimilarity between anemone species

One-way "within"				Two-way "within"			
Species	Enzymes	Av.Sq.Dist	Contrib%	Species	Enzymes	Av.Sq.Dist	Contrib%
A. sargassensis	SOD	0.0659	2.46	A. sargassensis	SOD	6.59E-2	2.46
	CAT	0.0674	2.51		CAT	6.74E-2	2.51
	LPO	0.714	**26.66**		LPO	0.714	26.66
	GR	0.786	**29.32**		GR	0.786	29.32
	GST	1.05	**39.05**		GST	1.05	39.05
B. canjicum	SOD	0.00492	0.49	B. canjicum	SOD	4.92E-3	0.49
	GST	0.128	12.7		GST	0.128	12.7
	LPO	0.183	**18.14**		LPO	0.183	18.14
	GR	0.252	**25.05**		GR	0.252	25.05
	CAT	0.439	**43.62**		CAT	0.439	43.62
A. bermudensis	GST	0.155	2.7	A. bermudensis	GST	0.155	2.7
	LPO	0.374	6.49		LPO	0.374	6.49
	CAT	0.45	7.82		CAT	0.45	7.82
	GR	0.743	12.91		GR	0.743	12.91
	SOD	4.04	**70.09**		SOD	4.04	70.09
B. caissarum	CAT	0.00449	0.47	B. caissarum	CAT	4.49E-3	0.47
	GST	0.00827	0.87		GST	8.27E-3	0.87
	SOD	0.0826	8.68		SOD	8.26E-2	8.68
	LPO	0.154	16.19		LPO	0.154	16.19
	GR	0.702	**73.78**		GR	0.702	73.78
A. Equina*	SOD	0.00497	0.05	A. equina*	SOD	4.97E-4	0.33
	LPO	0.0028	2.62		LPO	2.80E-2	2.52
	CAT	0.28	**26.14**		CAT	0.28	22.92
	GR	0.482	**45.03**		GR	0.482	43.06
A. sulcata	SOD	0.000631	0.06	A. sulcata	SOD	6.31E-4	0.06
	LPO	0.0163	1.54		LPO	1.63E-2	1.54
	CAT	0.0187	1.77		CAT	1.87E-2	1.77
	GST	0.865	**81.72**		GST	0.865	81.72
A.equina**	SOD	0.00703	0.62	A. equina**	SOD	7.03E-03	0.62
	LPO	0.0252	2.22		LPO	2.52E-02	2.22
	CAT	0.153	13.45		CAT	0.153	13.45
	GST	0.32	28.11		GST	0.32	28.11
	GR	0.633	**55.61**		GR	0.633	55.61

* control
**contaminated

The SIMPER (similarity percentages), within species, in each climatic coastal area showed that, for *A. sargassensis* the main contributions to dissimilarity were the GST (39.05%), GR (29.32&) and LPO (26.66%) enzymes activities. For *B. canjicum*, the main contributions to dissimilarity were CAT (43.62%), GR (25.05%) and LPO (18.14%) enzymes activities. For *A. bermudensis* the main contributions to dissimilarity were the SOD (70.09%) and GR (12.91%) enzymes activities. For *B. caissarum,* the main contributions to dissimilarity were GR (73.78%) and LPO (16.19%) enzymes activities. For *A. equina* the main contributions to dissimilarity were the GR (45.03%), GST (26.17%) and CAT (26.14%) enzymes activities. For *A. sulcata*, the main contributions to dissimilarity were the GST (81.72%) and GR (14.9%) enzymes activities.

3.4. Enzymatic activity vs species vs polluted/non polluted locations.

Results of the comparison from reference stations and from stations with different types of environmental contamination using the oxidative stress biomarkers are presented in Figure 2. Considering the different sampling stations, and without taking into account seasonal variations, in general the organisms from contaminated stations exhibited significantly lower mean enzymes activity levels than in reference stations (ANOSIM, Global R: 0.55, $p< 0.01$) (Table 4). Also it was revealed an interspecific variation in oxidative stress enzymes responses.

In respect to the Gluthathione Reductase (GR) average activities, in Tropical coastal areas, the higher value was observed in *B. cangicum* from reference site (11.5 ± 2.7, ITA, Itamaracá), and a lower value for *B. cangicum* from contaminated site (3.8 ± 1.7, OLI, Olinda). In the Subtropical coastal areas, the higher value was observed in *A. bermudensis* from reference site (9.7 ± 2 .8, PX, Punta Xen), and lower values in *A. bermudensis* for contaminated sites (4.9 ± 1.8, SIP, Siho Playa and NIT, Niteroi). An exception was observed in the Temperate coastal areas where the GR mean activity showed higher values for *A. equina* in the contaminated site (12.3 ± 2.7, PN, Praia Norte), and a lower value in *A. sulcata* in the reference site (7.7 ± 0.8, VPA, Vila Praia de Âncora).

Concerning The Catalase (CAT) average activities, in the Tropical coastal areas, a higher value was observed in *A. sargassensis* from reference site (123.8 ± 9.5, ITA, Itamaracá), and a lower value was in *B. cangicum* from contaminated site (40.8 ± 31.1, OLI, Olinda). In the Subtropical scenarios, a higher value was observed in *A. bermudensis* from reference site (87.45 ± 8.89 PX, Punta Xen), and lower values in *B. caissarum* for contaminated sites (14.1 ± 3.1, SIP, Siho Playa and NIT, Niteroi). In the Temperate coastal areas, the CAT mean activity showed a higher value in *A. equina* from reference site (56.6 ± 22.8, VPA, Vila Praia de Âncora), and a lower value in *A. sulcata* from the contaminated site (14.75 ± 2.3, PN, Praia Norte).

In respect to the Gluthatione S-transferase (GST) average activity, in Tropical coastal areas it was observed an exception to the normal pattern, where the higher value was observed in *A. sargassensis* from contaminated site (150.06 ± 55.8, OLI, Olinda), and a lower value in *B. cangicum* from reference site (46.58 ± 21.2, ITA, Itamaracá). In the Subtropical coastal areas, a higher value was observed in *A. bermudensis* from reference site (120.03 ± 17.9, PX, Punta Xen), and lower values in *B. caissarum* from the contaminated sites (19.58 ± 5.43, SIP, Siho Playa and NIT, Niteroi). In the temperate coastal areas, the GST average activity showed a higher value in *A. sulcata* from the reference site (172.77 ± 43.3, VPA, Vila Praia de Âncora), and a lower value in *A. sulcata* from the contaminated site (91.46 ± 30.9, PN, Praia Norte).

Taken into account the Lipid Peroxidation (LPO) average activity, in the Tropical coastal areas it was also observed an exception to the normal pattern, such as in the GST mean activity, where a higher value was observed in *A. sargassensis* from contaminated site (15.9 ± 5.2, OLI, Olinda), and a lower value in *B. cangicum* from the reference site (7.2 ± 2.6, ITA, Itamaracá). In the Subtropical coastal areas, a higher value was observed in *A. bermudensis* from reference site (8.51 ± 4.0, PX, Punta Xen), and a lower value observed in *A. bermudensis* from the contaminated sites (3.71 ± 2.1, SIP, Siho Playa and NIT, Niteroi). In the Temperate coastal areas, the LPO mean activity showed a higher value in *A. equina* from the reference site (3.4 ± 0.9, VPA, Vila praia de Âncora), and a lower value for *A. equina* from the contaminated site (2.61 ± 0.93, PN, Praia Norte).

In respect to the Superoxide Dismutase (SOD) mean activity, in the Tropical coastal areas, it was observed that the higher, in *A. sargassensis* (17977.4 ± 20606.5) and the lower values, in *B. cangicum* (5273.9 ± 4220), were both registered in the reference sites (ITA, Itamaracá). In the Subtropical coastal areas, the higher value was registered in *A. bermudensis* from reference site (33490.0 ± 9123.6, PX, Punta Xen), and a lower value in *A. bermudensis* from the contaminated sites (1507.0 ± 1251.3, SIP, Siho Playa and NI, Niteroi). In the Temperate coastal areas the SOD mean activity showed, an exception was also observed, such as in LPO and GST, where a higher value was registered, in *A. equina* from the contaminated site (4633.4 ± 5044.2, PN, Praia Norte), and a lower value registered in *A. sulcata* from the reference site (1507.0 ± 1251.3VPA, Vila Praia de Âncora).

ANOSIM (Analysis of similarities) testing identified significant differences in the multiple comparison pairwise results, between and within anemone species, in the oxidative stress biomarkers activities between species from reference and contaminated sites (Global R: 0.55, $p < 0.01$) (Tables 3 and 4). The *A. equina* and *A. sulcata* species appears to be the species that contributes to a better differentiation between reference and contaminated areas (ANOSIM one way and Two-Way Crossed Analysis, $p<0.01$).

SIMPER (similarity percentages) analysis between and within anemone species differentiates the enzyme activities contribution as being influential in differences from reference and contaminated areas (Table 5). The results showed that main contributions to dissimilarity between reference and contaminated areas were observed in *A. equina* and *A. sulcata* species, and the enzyme activities main contribution as being influential in the differentiation were the GR, CAT and GST enzymes activities (Tables 5 and 6), For the other anemone species enzymes activities contributions were as follows: *B. caissarum* due to the GR enzyme activities, *A. bermudensis* due to SOD, *B. canjicum* due to GR and LPO enzyme activities and *A. sargassensis* due to GST, GR and LPO enzymes activities (Tables 5 and 6).

4. DISCUSSION

For ecological risk assessment, predicting the effects of environmental perturbations requires biomarkers of short-term effects reflecting the mechanisms by which the health of organisms is altered (Cajaraville et al. 2000; David et al. 2002). Enzymatic activities are expected to play a significant role in conferring tolerance to such stressful and variable conditions upon these organisms. At the geographic scale organisms have developed an evolutionary capacity to tolerate environmental variability and changes by means of several mechanisms, such as behavioral adaptations, morphological alterations (intra and interspecific variation), regulation of reproduction, and cellular responses (Parmesan, 2006). These predictable responses to or unexpected changes in the environment can be regarded as stressful for organisms and seems to be crucial in their adaptation to highly fluctuating environments such as the marine environment. The results of the variations in the oxidative stress biomarkers levels revealed biological effects induced by pollutants under the influence of different climatic marine scenarios. The results of SIMPER differentiate significant enzyme activities contribution as being influential in the three coastal areas. For tropical areas the main contributions were due to GST, GR and LPO activities. For sub-tropical areas the main contribution was due to SOD and GR activities. For temperate areas the main contributions were due to GST and Gr activities. Glutathione-S-transferases (GST) is considered a family of cytosolic enzymes important in the process of xenobiotic detoxification compounds of endogenous since catalyses the combination of compounds electrophilic, resulting from the phase I of biotransformation, with the glutathione which is essential in the maintenance of normal physiological processes (Hayes and Pulford, 1995; Livingstone et al. 1995). SOD has particular value as an antioxidant that can help to protect against cell destruction. It is responsible both for the direct damage of biological macromolecules and for generating other reactive oxygen species. The substrate of superoxide dismutases (SODs) is the superoxide radical anion (O2 -) which is generated by the transfer of one electron to molecular oxygen. SODs keep the concentration of superoxide radicals at low levels and therefore play an important role in the defense against oxidative stress (Fridovich, 1997). Lipid Peroxidation (LPO) is usually considered in pollution monitoring studies (López-López et al. 2011; Bocchetti and Regoli, 2006) as indicative of oxidative stress damage, a consequence xenobiotic enhanced metabolism and/or metal exposure (Livingstone et al. 1990; Valavanidis et al. 2006). It integrates the noxious effects caused by oxyradical species that cannot be counteracted by the natural oxidative stress defenses with the consequent membrane destabilization and membrane-bound enzymes (Porter and Zhisheng, 1995).

The results of PERMANOVA revealed that the enzymatic activities of the anemones species showed significantly differences and was influenced by the climatic shift. It was also observed significant differences between all enzymatic activity vs species. Organisms respond to environmental changes by regulating metabolic pathways to prevent physiological damage. The physiological performance which reflects their evolutional adaptation to local environment (Dong et al. 2011; Somero, 2012), provide a direct link between thermal physiology and animals ecology, which elucidates the role of aerobic metabolism in the thermal tolerance across latitudes (Somero, 2012). The selected species revealed widely different thermal tolerances and adaptive differences in their enzymatic responses. An advantage afforded by these species was that the differences observed in the enzymatic responses are likely to indicate adaptation due to selective pressures. This arises from different thermal environments during their recent separate evolutionary histories rather than merely reflecting phylogenetic distance (Hellberg, 1998). The results showed that the selected sea anemones species, directly exposed, at different latitudes and ecoclimatic regions, to extreme environmental conditions, presented consistent oxidative stress responses related with physiology alterations. The individual expressions precede population-level changes and are useful indicators if linked to specific physiological or ecological events (Dong et al. 2011 and Schroth et al. 2005). The degree of response seems to differ among species in relation to trophic level that they occupy, their habitat type, feeding habits, biotransformation capabilities and abiotic factors (Lee and Anderson, 2005; Barreira et al. 2007).Their habitats includes environments which are continually exposed to garbage dumping, untreated sewage inflow, land and river runoff, atmospheric fallout from heavy traffic and various small-scale industries scattered in coastal areas. *A. equina* is an intertidal specialist that reaches its highest densities on wave-cut platforms on the lower portion of shores. The intertidal habit is subject to waves and surge, the water being laden with sand, stones and debris, as well as containing planktonic, nektonic and benthonic animals that can be dashed against the rocks. *Actinia* sp. diet is heavily influenced by shore height and wave exposure, but also that *A. equina* scavenges (rather than preys) on the larger food items that it ingests were known to take up dissolved organic material, to assimilate energy from bacteria and microalgae (van Praët, 1985), and to predate on a range of organisms (e.g. Chintiroglou and Koukouras, 1992, Kruger and Griffiths, 1998). *A. sulcata* is an example of a cnidarian that has strong regenerative abilities and employs asymbiotic relationship with photosynthetic zooxanthellae. It is commonly found on lower shorelines and sea beds in temperate regions (Muller-Parker and Davy, 2001). Green and brown colour morphs of this species exist and it is thought that the colour difference may be due to the presence of green fluorescent protein and pink chromoproteins. *A. bermudensis* and *A. sargassensis* inhabits shallow waters of the lagoon reef zone in places where there are no direct wave action but they are also found under stones and coral gravel, In tide pools and under rocks, permanently submerged in the pools, not suffering desiccation, *B. caissarum*, a Brazilian endemic species, occurs along the mainland coast although dense groupings are only found on firm substrata in the infralittoral zone along open coastlines or in sheltered bays and coves in the mid-littoral zone, where they may occur on semi-stable substrate. Most commonly found in the mid-littoral zone inside shady sea-caves and rarely in tide-pools (Amaral et al. 2000). *B. cangicum* inhabits shallow rock pools formed at low tide in the flats of the beach rock, where: there was no direct wave action. One of these adaptations is its aggressive capacity due to the large size and the presence of marginal spherulae, and associated the larger size with the low rate of respiration of anemones in protected zones compared with exposed zones. In this way individuals conserve more energy which can then be channeled into greater growth. The behaviour in some individuals of distending their tentacular crown into the remnant water has been described previously by Belém and Preslercravo, 1973 and seems to be related to food catch, the presence of light, sea tides and avoidance of desiccation during low tides. All these mechanisms presented by *B. cangicum* justify its great distribution along the Brazilian coast.

Consistent differences between contaminated and non-contaminated sites were evident at all selected species, both in and in significant pairwise comparisons in the randomisation tests ANOSIM and SIMPER (Analysis of similarities) identified significant differences in the multiple comparison pairwise results, between and within anemone species, in the oxidative stress biomarkers activities between species from reference and contaminated sites The *A. equina* and *A. sulcata* species appears to be the species that contributes to a better differentiation between reference and contaminated areas. SIMPER (similarity percentages) analysis between and within anemone species differentiates the enzyme activities contribution as being influential in differences from reference and contaminated areas. The results showed that main contributions to dissimilarity between reference and contaminated areas were observed in *A. equina* and *A. sulcata* species, and the enzyme activities main contribution as being influential in the differentiation were the GR, CAT and GST enzymes activities. It has been described that clearly differences between symbiotic and aposymbiotic anemones related to its responses to contamination, due to possible differential contaminants accumulation by the algal symbionts (Mitchelmore et al. 2002). A common mechanism employed to exclude and control contaminants entry is the binding of contaminants by external mucus coatings, Symbiotic anemones appeared to produce more mucus than aposymbiotic ones. The sequestration of metals into granules has been demonstrated in cnidarian, process that may act in conjunction with mucus production such that metal granules are concentrated in mucus and sloughed off. *A. equina* and *A. sulcata* are also examples of cnidarians that has strong regenerative abilities and employs asymbiotic relationship with photosynthetic zooxanthellae, that could play an important role to contaminants responses (Merle et al. 2007).They are commonly found on lower shorelines and sea beds in temperate regions (Muller-Parker and Davy, 2001). Green and brown colour morphs of this species exist and it is thought that the colour difference may be due to the presence of green fluorescent protein and pink chromoproteins. Studies investigating the bleaching phenomenon and symbiotic relationships in corals use *A. sulcata* and *A. equina* as models (Richier et al. 2006; Merle et al. 2007). It has been shown that *A. equina* and *A. sulcata* contains antioxidant enzymes including glutathione peroxidase (Hawkridge et al. 2000) and demonstrates strong antioxidant responses. Recent works (Childs, 2013) report that exposure to copper caused significant bleaching of zooxanthellae in *A. sulcata* as well as significant increases in glutathione content, but there was a mixed response in levels of lipid peroxidation products. Environmental stress such as metal exposure and hyperthermia causes reactive oxidation species (ROS) to proliferate in the photosynthetic regions of the zooxanthellae, which can then trigger the elimination of symbionts from the host (Venn et al. 2008). Elevated levels of ROS are also thought to induce the up-regulation of antioxidants such as glutathione (GSH) in order to reduce cellular damage (Halliwell and Gutteridge, 2007; Lushchak, 2011). For the other anemone species enzymes activities contributions were as follows: *B. caissarum* due to the GR enzyme activities, *A. bermudensis* due to SOD, *B. canjicum* due to GR and LPO enzyme activities and *A. sargassensis* due to GST, GR and LPO enzymes activities Considering the different sampling stations, and without taking into account seasonal variations, in general the results showed that the organisms from contaminated stations exhibited significantly lower mean enzymes activity levels than in reference stations Also it was revealed an interspecific variation in oxidative stress enzymes responses. Due to the different thermal environments exposition during their recent separate evolutionary histories and because they are sedentary animals that perform low rates of movement these organisms might be subject to consistent differences in microclimate and have other suitable characteristics that drive differences in behaviour and stress responses (Cajaraville et al. 2006; Marigómez et al. 2006).

The RDA output indicated associations between lipid peroxidation and CAT activities with water temperature. These biomarkers, also, revealed lowest activities in organisms collected in areas with higher oxygenated waters. Therefore these results may suggest the lower oxygenated water, associated with anthropogenic impacts may be responsible for the enhancement of antioxidant system. Overall results suggest SOD as the main discriminant biomarker, followed by CAT and LPO. The selected biomarker responses, observed in organisms captured along the three climate zones, indicate that *A. sargassensis, A. bermudensis* and *A. equina* revealed to be the most sensitive organisms in the tropical, subtropical and temperate areas, respectively. The results of sea anemones enzymatic responses approach in detecting differences among contaminated sites as the advantage of adopting lower taxonomic discrimination in the determination of perturbation in benthic communities. This appealing for cost and thus management reasons, and may thus reach a compromise between management and scientific objectives in the selection of appropriate species to discriminate in impact assessment programs.

5. CONCLUSIONS

The present study demonstrates the antioxidant enzyme responses in six sea anemones species, *Actinia equina, Anemonia sulcata* (Temperate climate, Portuguese coast), *Anemonia sargassensis, Actinia bermudensis*, *Bunodosoma caissarum* and *Bunodosoma cangicum* (Tropical climate, Brasilian and Sub Tropical climate, Mexican coast), marine organisms that are directly exposed, at different latitudes and ecoclimatic regions, to extreme environmental conditions, such as contaminated exposure, which may cause physiology alterations. The results showed that these sea anemones species have a number of properties that make them useful sentinels for environmental monitoring and can provide a measure of environmental pollution with observable cellular and physiological responses.

Oxidative stress is emerging as a common theme in connection with the impact of global climate change (e.g., global warming and ozone depletion) on natural ecosystems at all trophic levels. These stress biomarkers responses may be considered as indicators of the health of the related ecosystems. The increases in these enzyme activities showed the overproduced ROS level in the metabolism. However, more investigations are still needed in order to obtain a complete picture of the combined effects of chemical and forced natural factors such as climatic shifts on the cellular pathways of sea anemones enzymatic responses

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CHAPTER 5

Physical Stressors Effects on Natural Populations

Chapter 5. Article 4: Status submitted on Aquaculture

Temperature tolerance test exposition with temperate sea anemone *Actinia equina,* a climatic and environmental changes simulation

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ABSTRACT

Atlantic and Mediterranean warming-related diseases outbreaks and species shifts recently been documented. Evaluated tools of short-term effects on the health or organisms resistance are necessary to assess and understand mechanisms affecting marine biodiversity. Until now, climate warming been studied at population or community level. Here we offer a better understanding of such phenomena at the organism level, using anatomic-morphological approaches to interpret effects of natural physical stressors, according to behavioral patterns. The main goal of this work was to evaluate the sea anemones temperature tolerance. This study takes a behavioral (morphological and anatomic parameters, with physiological implications) to identifying changes soon after exposure to the physical stressors temperature (10, 15, 20, 25 and 30ºC) in the temperate sea anemone *Actinia equina* over 96h of exposure, as an early warning signalizing. Condition index, reproduction and behavior endpoints were assess. Behavioral patterns analysis placed the differentially ecological functions in a wide range of categories including tentacle flexion, tentacle retraction, column cavitation, peristome depression and oral disc flexion. This suggests that the early stress response to elevated temperature involves essentially all aspects of same chemical reactions, in this case we observed an receptors functioning and the frequency of open-close oral sea anemones, tentacles and columns anatomic alterations to detect earlier the effects of physical stress induction. The hypothesis tested was that key species react to different temperature ranges in order to demonstrate that species from different climatic zones could have the same behavioral pattern but have intrinsic adaptations on each climatic zone. The results of these study, suggest that water nutrients availability, reproductions rate (Number of polyps), survival (Condition index) and temperature variances were significantly on behavioral answers.

Key Words: Temperature Tolerance, Behavior, Early Warning, Climatic Changes.

INTRODUCTION

The coral reefs community is a very rich on biodiversity. Many studies reveal that organism inhabit the reefs are very sensitive to environmental alterations. It has been over 10 years since the phenomenon of extensive coral bleaching first described. In most cases, bleaching been attributed to elevated temperature, but other instances involving high solar irradiance, and sometimes disease, have also been documented.

Massive bleaching events are becoming an increasingly important cause of mortality and reef degradation on a global scale, linked by many to global climate changes. Previous studies assessing the effects of bleaching on natural populations and laboratorial exposures have investigated the effect of environmental and physical stressors, such as temperature and UV (ultraviolet) radiation, on symbiotic algae (zooxanthallae) (Lesser, 1997).

The present study focused only on responses of sea anemones to the environmental stress temperature, both above and below the optimum temperature. Increased temperature been shown to decrease the photosynthetic performance of zooxanthellae or cause its release, as well as to cause oxidative stress in anemones and consequently bleaching (Lesser, 1997; Perez et al, 2001; Richier etal, 2006; Bhagooli and Hidaka, 2004).

In addition, Programmed Cell Death (PCD) and necrosis found to occur simultaneously in both host tissues and zooxanthellae (Dunn et al, 2002). Moreover, these effects depend on the temperature and duration (Dunn et al, 2002).

Low temperatures can also have severe effects on anemones, which depend on the temperature and duration (Muscatine, 1991). Decreased temperatures were suggest to cause exocytosis of zooxanthellae (Muscatine, 1991; Gates et al, 1992).

However, the behavior responses of anemones to temperature-induced bleaching remain largely unknown. In this study, the behavior responses of a temperate sea anemone was assessed using criteria that had previously been validated for sea anemones as indicators of environmental and climate changes.

Anatomic and morphological parameters of physiological alterations were assessed, namely tentacles flexion, retraction, oral disc flexion, column cavitation and peristome depression. Clark and Kimeldorf (1971), who studied behavioral reactions of the sea anemone *Anthopleura xanthogrammica* to ultraviolet and visible radiations, first described these parameters. Each behavioral endpoint has a physiological implication and ecological Interpretation. It is important to study the response of a wide variety of anemones to temperature stress, because anemones can have different responses to temperature, even if they are genetically similar, as shown by Walsh and Somero (1981). These authors reported that anemones from southern and northern California (USA), despite being genetically similar (electrophoretic method), had different oxygen consumption patterns in response to acclimation and acute changes in temperature. The two populations also differed in the extent of metabolic compensation to temperature following several weeks of acclimation (Walsh and Somero, 1981). In this study, we used the temperate sea anemone *Actinia equina,* collected in the northwest of Portugal, near the border with Spain. This sea anemone is a cosmopolitan species, very common on the Iberian coast and possesses physiological adaptations to body water balance (Gadelha et al., 2013). Portugal is the southern geographical limit for many boreal species and the northern or western limit of subtropical and Mediterranean species (Saldanha, 1974).

In opposite, a few works was developed concern the environmental stressors sea anemones directly effects it was made. It is a fact that Temperature variations consist in an important key to understand marine and environmental coastal alterations and climatic changes. Sea anemones and another cnidarians groups (such as corals), are a good vehicle to study and understood the natural and modified environmental processes (global temperature increase) and the effects. A recent and innovative goal was to try anticipating these alterations and to create a database to prevent and predict the environmental and climatic changes before the damage was irreversible.

Bae and Park (2014), recently reviewed the development and application of BEWS (Biological Early Warning systems) using various groups of organisms (such as bacterial, algae, cladocerans, bivalve and fish) and computational methods to process the behavioral monitoring data. Thus, in this work we assessed the temperature tolerance of *Actinia equina* ending to assess whether it is or not a suitable species for BEWS. The hypothesis tested was that key species react to different temperature ranges in order to demonstrate that species from different climatic zones could have the same behavioral pattern but have intrinsic adaptations on each climatic zone. Based on these affirmations, the sea anemones could be earlier environmental signalizing to climatic changes. The present study intends to add a new species into this baseline, using a benthonic key-species and a cosmopolitan sea anemone: *Actinia equina*.

The goal of this work was to investigate behavioral responses to temperature ranges of the anemone species *Actinia equina* that be separated into reactions having markedly different temperature/time dependence. The following behavioral parameters assessed tentacle flexion, tentacle retraction, oral disc flexion, column cavitation, and peristome depression, each one with ecological interpretations and physiological implications. We simulated temperature stress, simulating a short-term steadily rising of background sea temperature, to which corals and other benthonic species might be exposed in the future. This study is timely, in view of our concern about worldwide coastal areas condition and the effects of climate change. We will study knowledge of physical, toxicological and ecological factors involved in benthonic organism's damages, the mechanisms of species richness low and environment transformation, and the ecological consequences for coastal organism communities.

MATERIAL AND METHODS

Sampling site

The sampling site chosen based on common distribution of anemones on the Portuguese coast. The site is located in the NW Portuguese coast, specifically in Vila Praia de Âncora (41°49'13.54"N and 8°52'26.05"W). This site is situated near small fishery villages and far from big population aggregates and potential sources of contamination (agricultural, urban and harbor). Several studies performed in the Portuguese coast indicated this site as relatively undisturbed by anthropogenic pressures and it been used as a reference site in previous studies (Moreira et al., 2005).

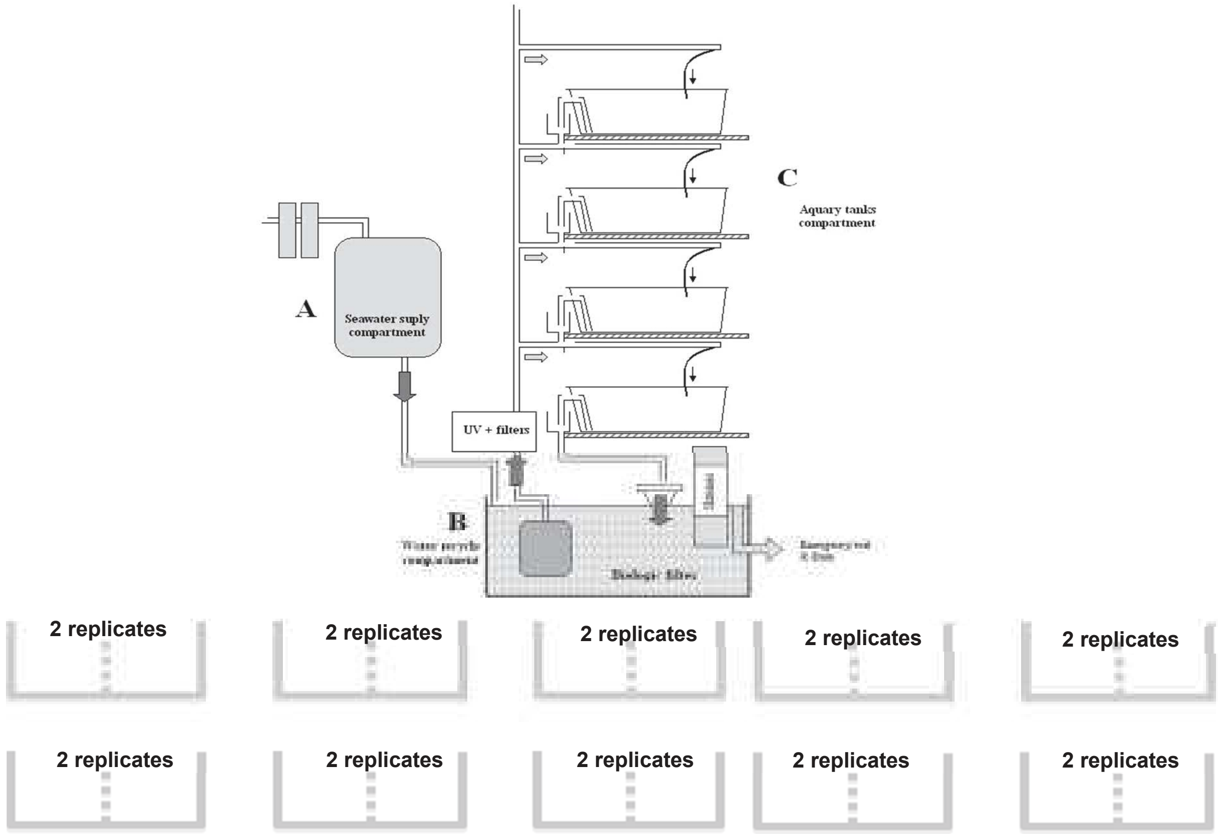
Animal sampling

Identification of anemones performed using the Handbook of the Marine Fauna of the North-West Europe (Cornelius, 1995). One hundred *Actinia equina* specimens were collected by hand and with the aid of mussel shells (Zelnio et al., 2009) during low tide, avoiding to collect individuals similarly colored in the vicinity, since these could be clones (Scott and Harrison, 2009). The organisms collected were 1-3 cm in length. One hundred specimens were catch and transported to the lab facilities in buckets with oxygenated seawater.

The following abiotic factors were measured in situ at the site, in the rainy season in the year of 2012: salinity ($g.L^{-}1$) and conductivity ($mS\ cm^{-1}$) (Wissenschaftlich Technische Werkstätten – LF 330 meter, Brüssel, Belgium), dissolved oxygen (percentage) saturation (Wissenschaftlich Technische Werkstätten Cell Ox 325) and pH (Wissenschaftlich Technische Werkstätten 537 meter).

Acclimation in a flow-through system and experimental design

After arrival to the lab, anemones transferred to 2.5 L aquaria with artificial seawater in a flow-through system, for depuration. The following conditions were kept temperature (20 ± 1.8 ºC), salinity (32 ± 0.3 $g.L^{-1}$), pH (7.74±0.16), dissolved oxygen (84.14 ± 6.33 %) and photoperiod 16h light: 8h dark. Artificial seawater was prepared with MilliQ® complemented with salt "Instant Ocean Synthetic Sea Salt" (Spectrum Brands, USA) and adjusted for pH. Anemones kept at 20ºC since it is the recommended temperature for temperate scenarios (USEPA, 1994). Anemones feed every three days with feed diet fish, a standard diet with a low lipid concentration, to avoid the excessive lipid accumulation. Organisms kept under these conditions during two-weeks for acclimation ("personal communication" Gadelha *et al*, 2010). During this period, the water parameters checked regularly, the organism's density adjusted to four individuals in each aquarium, and survival performance and reproduction rate verified. After the two-week acclimation period, organisms were acclimated to the laboratory conditions during four weeks approximately ("personal communication" Gadelha et al, 2010; 2011). The conditions were very similar to the described above for depuration but included a biological filter. The biological filter consisted of natural seawater, including microorganisms, which introduced in the flow-through system, improving the water quality. The figure 1 illustrate the flow-through system. The experiments preparation reveal the priority of efficiency of acclimation in the flow-through system during four weeks to water quality and organisms preparation to the temperature tolerance tests. The sea anemone *Actinia equina* viability, growth, behavior and mortality analyzed to answer the temperature tolerance variations Table 1 illustrate all controlled conditions during the acclimation (Figure 1).



T°= 20°C

Tank number = 10 Replicates /tank = 2 Total samples = 20

Time-course = endpoint after 96 hours

Figure1: The flow-through system used to acclimatizing the sea anemones *Actinia equina* and experimental design *(Abreu, S.N)*.

Experimental design

The experimental procedures were developed following the maintenance guidelines (USEPA, 1994) with minor adjustments for sea anemones toxicological tests (Gadelha et al 2010, 2012) (see Tab 1).

Table 1: Acclimation conditions previous the physical stressors exposition tests.

Parameters	Conditions
System	continuously
Salinity	32 ± 0,29
Temperature	20 ± 1,75
pH	7,74 ± 0,16
Dissolved Oxygen (%)	84,14 ± 6,33
Photoperiod	16:8 (Artificial light)
Type of Aquarian	glass, transparent, with cover
Type of Water	Artificial seawater (MiliQ® with marine salt)
Water Volume	2,5 L (each Aquarian)
Water change	continuously
Aeration	continuously
Nº of *Actinia equina (adult)*	100
Nºof *Actinia equina*	100
Number of Replicates (each Aquarian)	*2*
Diet	Each 3 days *
Monitoring	Daily (each 6 hours)

*Feed diet fish

* Artificial Food composition: Protein = 55.00%, Oil =14%, Ash =12%, Fibre =1%, Vit A =30,000 i.u/Kg, Vit D3 =2,500 I.U/Kg, Vit E =700mg/kg, Vit C =2,000mg/kg, W3 HUFA =30 mg/g dwt.

Groups of two specimens incubated in 2.5 L aquaria containing artificial seawater, under continuous aeration and the above-described conditions and expose to the test temperature: 10, 15, 20, 25 or 30ºC for 96 h, which represents the exposure period to different subtidal temperatures. Tests were carried out in duplicate samples per aquaria, with 10 Aquarians (n=20 for each treatment). All other factors (e.g. 32 ± 0.29-ppm salinity, pH 7.74 ± 0.16, and dissolved oxygen 84.14% ± 6.33) were identical in all the experimental groups.

During the test period survival, reproduction and behavior of the anemones analyzed on the second and last day. Reproduction was assessed based on detachment of juvenile polyp's (asexual reproduction), which were counted in each aquarium. Anemones behaviour assessed based on tentacle flexion, tentacle retraction, oral disc flexion, column cavitation and peristome depression. For each parameter, a categorical value was registered: flected, semi-flected and extended.

Moreover, the condition index was determined at the end of the experiment. The condition index was determined based on the fresh weight of the organisms, using the following equation. Jordi and Green (2009) also present an empirical and theoretical comparison of the scaled mass index and OLS residuals as CIs. They argue that the scaled mass index is a useful new tool for ecologists.

$$CI = \frac{Wf}{Wi}$$

Where CI is the condition index, W_f is the final weight and W_i is the initial weight (at the beginning of the experiment). Anemones wet weight was determined to the nearest 0.01 mg.

Chemical analysis

Water physicochemical parameters (temperature, pH, salinity, conductivity and dissolved oxygen), chlorophylls and nutrients analyzed on the first and last day for each treatment. Water samples for analysis of both chlorophylls and nutrients water were collected from the bottom of the aquaria, since chlorophylls are photosensitive, thus an easily degradation material. Water samples for nutrients (nitrites, nitrates and phosphates) were previously filtered with glass fiber filters (Whatman, GFC, 1.2 μm) and then analyzed using the Diazotization Method, Cadmium Reduction and Ascorbic Acid Method and USEPA method using powder pillows, respectively (Eaton, 2004). Water samples for chlorophylls (a, b and c) were filtered with glass fiber filters (Whatman GF/C, 1.2 μm) and analyzed following the Jeffrey & Humphrey`s tri-chromatic Equations (Jeffrey & Humphrey, 1975 *in* APHA, 1999).

Statistical analysis

Statistical analysis performed using SPSS v. 20 (IBM, 1989). Data was tested for normality (Kolmogrov-Smirnov test) and homogeneity of variances (Barlett test), following Zar (1996). For data normally distributed, a repeated-measures ANOVA was used to assess fluctuations of condition index, physical-chemical parameters, chlorophylls (a,b and c) and nutrients in each aquarium along time. Multiple comparisons relative to the control (20ºC) performed using the Tukey and Dunnet to the parametric data and Kruskal wallis, Dunn`s for the non-parametric data test and Student Newman Keuls was improved too. Results of the behaviour endpoints constituted non-parametric data and the statistical analysis performed with the Kruskall-Wallis test followed by the multiple comparisons in relation to temperature and time. Multiple comparisons performed using the 20°C treatment as the control. The Spearman`s correlations was employed to identify the possible correlations between behavior parameters and temperature/time variation. All statistical analysis based on a 0.05 significance level.

RESULTS

1- Physico-chemicals parameters

The water physicochemical parameters in each treatment are depict in Figure 2. There were significant differences among temperatures, but they are not ecologically relevant. The coefficient of variation calculate in order to show the variation level of each parameters, represented on Table 2.

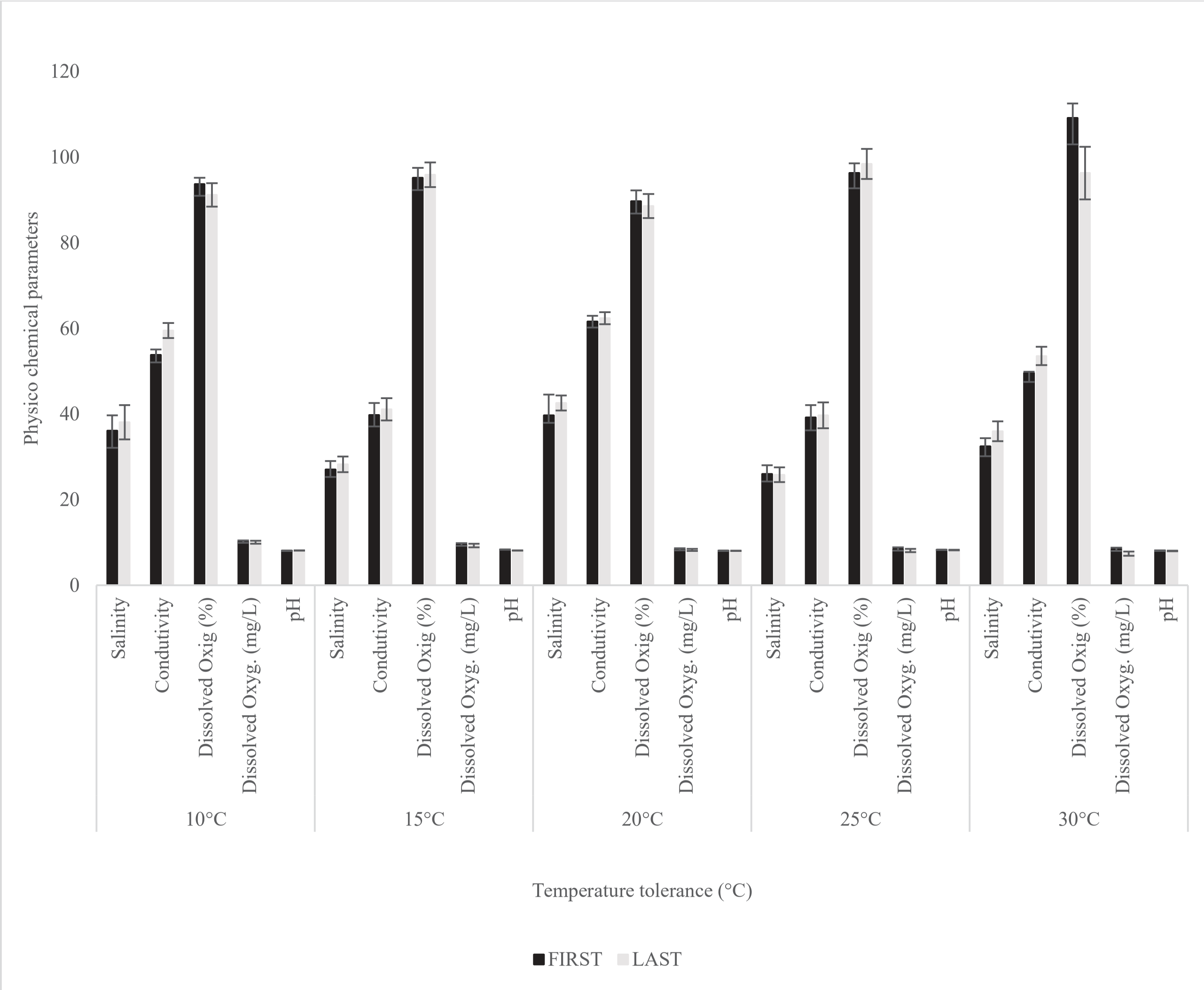


Figure 2. Physico-chemical parameters on each temperature tolerance test.

Table 2. Coefficient of variation values for each Physico chemical parameters variation with temperature.

Temperature (°C)	Parameters	**CV	
		First day	Last day
10°C	Salinity	0.099	0.104
	Condutivity	0.022	0.029
	Dissolved oxygen	0.015	0.030
	pH	0.003	0.004
15°C	Salinity	0.072	0.064
	Condutivity	0.072	0.063
	Dissolved oxygen	0.024	0.030
	pH	0.003	0.004
20°C	Salinity	0.122	0.041
	Condutivity	0.021	0.022
	Dissolved oxygen	0.028	0.032
	pH	0.004	0.003
25°C	Salinity	0.079	0.067
	Condutivity	0.074	0.075
	Dissolved oxygen	0.023	0.035
	pH	0.006	0.007
30°C	Salinity	0.056	0.064
	Condutivity	0.005	0.039
	Dissolved oxygen	0.031	0.064
	pH	0.005	0.008

**Coefficient of variation

Dunnett analysis for Physic chemical parameters variation vs Temperature is significantly for all parameters within and between, p = 0 (see Table 3). Dunnet analysis for PFQs vs times showed that dissolved oxygen and pH parameters varied along the 96h tests (p = 0.02 and 0.009, respectively).

Table 3. Multiple comparison between Physico chemical parameters and temperature tolerance tests.

Multiple Comparisons

Dunnett t (2-sided)

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Salinity	10°C	30°C	2.85000*	.94072	**.011**	.5137	5.1863
	15°C	30°C	-6.56500*	.94072	**.000**	-8.9013	-4.2287
	20°C	30°C	6.90000*	.94072	**.000**	4.5637	9.2363
	25°C	30°C	-8.33500*	.94072	**.000**	-10.6713	-5.9987
Condutivity	10°C	30°C	5.08500*	.83560	**.000**	3.0098	7.1602
	15°C	30°C	-11.18000*	.83560	**.000**	-13.2552	-9.1048
	20°C	30°C	10.42000*	.83560	**.000**	8.3448	12.4952
	25°C	30°C	-12.15500*	.83560	**.000**	-14.2302	-10.0798
Dissolved Oxygen %	10°C	30°C	-10.28500*	1.39207	**.000**	-13.7422	-6.8278
	15°C	30°C	-7.20000*	1.39207	**.000**	-10.6572	-3.7428
	20°C	30°C	-13.60000*	1.39207	**.000**	-17.0572	-10.1428
	25°C	30°C	-5.39000*	1.39207	**.001**	-8.8472	-1.9328
Dissolved Oxygen mg/L	10°C	30°C	2.22600*	.13723	**.000**	1.8852	2.5668
	15°C	30°C	1.49000*	.13723	**.000**	1.1492	1.8308
	20°C	30°C	.39500*	.13723	**.018**	.0542	.7358
	25°C	30°C	.35600*	.13723	**.038**	.0152	.6968
pH	10°C	30°C	.04050	.02294	**.237**	-.0165	.0975
	15°C	30°C	.15250*	.02294	**.000**	.0955	.2095
	20°C	30°C	.00700	.02294	.994	-.0500	.0640
	25°C	30°C	.17700*	.02294	**.000**	.1200	.2340

*The mean difference is significant at the 0.05 level.

The only parameters that not verified variation it was pH in 20°C and 30°C (p =0.99). Tukey analysis for Physic chemical parameters variation vs Temperature is significantly different in almost possible combinations, see table 2. Tukey analysis for physic chemical parameters vs time only showed significantly variation between dissolved oxygen and pH (p = 0.02 and 0.009) (Table 4).

Table 4. Tukey analysis results to comparison Physico chemical parameters vs temperature variation.

Tukey HSD			
Dependent Variable	(I) Temperature	(J) Temperature	Sig.
Salinity (psu)	10°C	15°C	0
		20°C	0
		25°C	0
		30°C	0.026
	15°C	10°C	0
		20°C	0
		30°C	0
		10°C	0
	20°C	15°C	0
		25°C	0
		30°C	0
		10°C	0
	25°C	20°C	0
		30°C	0
		10°C	0.026
		15°C	0
	30°C	20°C	0
		25°C	0
		15°C	0
		20°C	0
Condutivity (µs/cm)	10°C	25°C	0
		30°C	0
		10°C	0
		20°C	0
	15°C	30°C	0
		10°C	0
		15°C	0
		25°C	0
	20°C	30°C	0

		10°C	0
		20°C	0
		30°C	0
	25°C	10°C	0
		15°C	0
		20°C	0
		25°C	0
	30°C	25°C	0.006
		30°C	0
		20°C	0
		15°C	0
		25°C	0
Dissolved Oxygen %	10°C	30°C	0
		10°C	0.006
		20°C	0
	15°C	30°C	0.002
		10°C	0
		15°C	0
		20°C	0
	20°C	25°C	0.002
		15°C	0
		20°C	0
	25°C	25°C	0
		30°C	0
		10°C	0
		20°C	0
	30°C	25°C	0
		30°C	0
		10°C	0
		15°C	0
Dissolved Oxygen mg/L	10°C	30°C	0.039
		10°C	0
		15°C	0
		10°C	0
	15°C	15°C	0
		20°C	0.039
		15°C	0
		25°C	0
	20°C	10°C	0
		20°C	0

		30°C	0
		15°C	0
	25°C	25°C	0
		10°C	0
		20°C	0
		30°C	0
	30°C	15°C	0
		25°C	0
pH	10°C	15°C	0
		25°C	0
		10°C	0
	15°C	20°C	0
		30°C	0
	20°C	15°C	0
		25°C	0
	25°C	10°C	0
		20°C	0
		30°C	0
	30°C	15°C	0
		25°C	0

*The mean difference is significant at the 0.05 level.

The amount of chlorophylls *a*, *b* and *c* in the water was not significantly affected by temperature (Tukey and Dunnet, df = 4, α= 0.05).

The variation of nutrients (nitrites, nitrates and phosphates) among test temperatures shown in Fig 3. Significant differences relative to the control were found for nitrites, nitrates and phosphates (test Tukey, df = 4, p = 0.004), nitrates/ phosphates and phosphates/ nitrates (test Tukey, df = 2, p = 0) (Table 5).

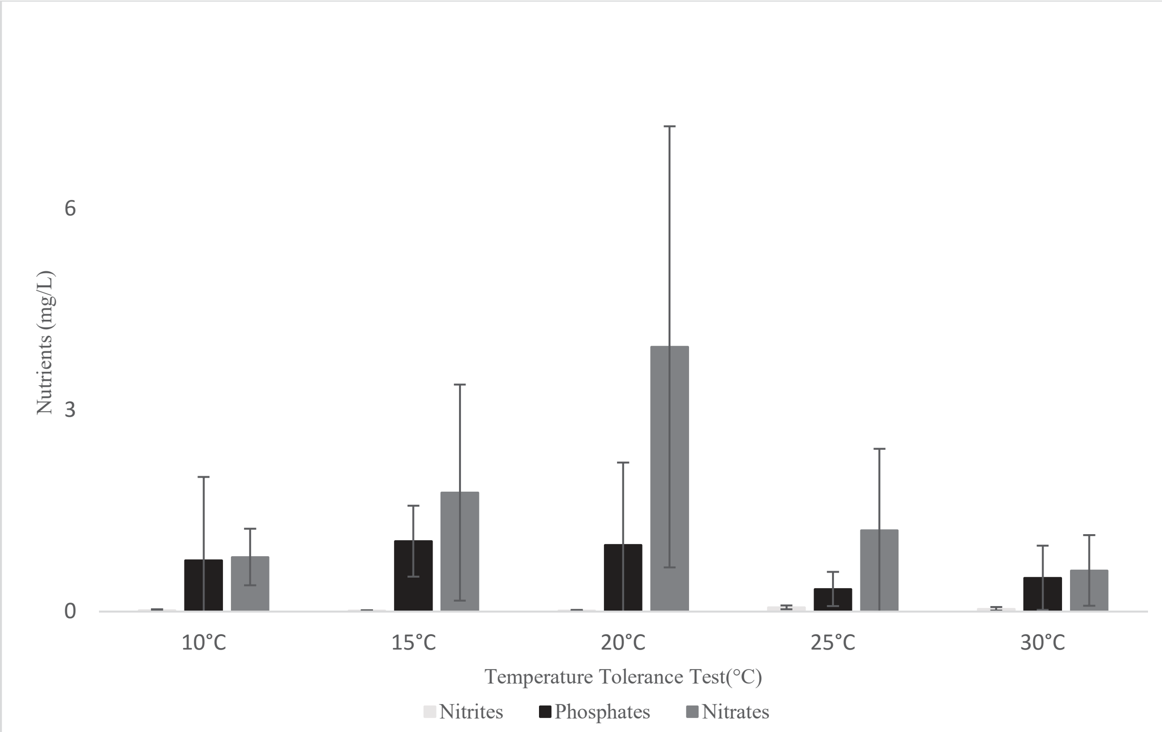


Figure 3. Nutrients on each temperature tolerance test.

The Tukey test showed the significant nutrients varied when compared 10°C with 20°C and 20°C with 25°C and 30°C ($p = 0.01$, 0.02 and 0.05, respectively) (Table 5).

Table 5. The Tukey HSD results to nutrients variations between each other.

Multiple Comparisons

Dependent Variable Nutrient value

Tukey HSD

(I) Nutrient type		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Nitrites	PH**	-.69778*	.25887	.021	-1.3107	-.0848
	NA**	-1.63898*	.25887	.000	-2.2519	-1.0260
Phosphates	NI**	.69778*	.25887	.021	.0848	1.3107
	NA	-.94120*	.25887	.001	-1.5541	-.3283
Nitrates	NI	1.63898*	.25887	.000	1.0260	2.2519
	PH	.94120*	.25887	.001	.3283	1.5541

*. The mean difference is significant at the 0.05 level. **PH, NA and NI- Phosphates, Nitrates and Nitrites, respectively.

2- Physiological endpoints

Survival was 100% throughout the test period, except for anemones exposed to 30ºC. At this temperature, survival reduced to 55% on day 4.

Reproduction was null after 48 h of exposure, for all temperatures. After 96 h of exposure, it was higher at 10ºC and lower at 25ºC (Tab 6).

Table 6. Survival and Reproduction of adult polyps on each temperature test tolerance, on first and last observation, along 96 hours.

	Temperature									
	10°C		15°C		20°C		25°C		30°C	
Time	Second day	Last day	Second day	Last day	Second day	Last day	Second day	Last day	Second day	Last day
Survival	20	20	20	20	20	20	20	20	20	11
Reproduction	0	10	0	7	0	2	0	0	0	7

The reproduction, measured as the number of polyps released during 4 days, was significantly affected by temperature (H = 15.59, df= 4, $p = 0.004$) (Figure 4). The results for a n° of polyps on each temperature after the 96 hours observed significant differences $p = 0.004$ and the Dunn`s Test tested and the result founded significantly differences between 10° and 25°C (Figure 4).

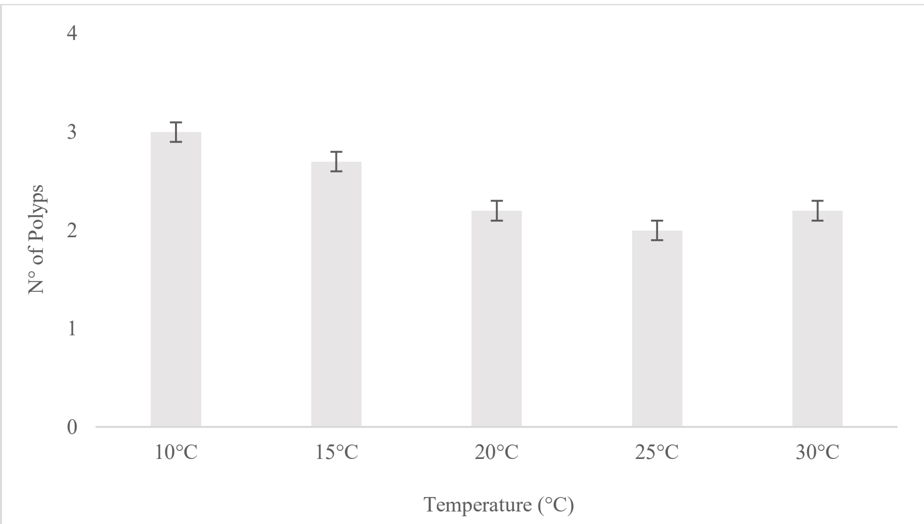


Figure 4. Release of juvenile polyps per groups of 2 anemones (each replicate in 10 aquaria, $N_o = 20$) exposed to 10, 15, 20, 25 and 30ºC during 96h, with a N_f. Error bars represent standard error. N_o and N_f, number of individual at the first day and the last day, respectively.

The Figure 5 show the Condition Index values on temperature test tolerance during 96h. Dunn`s test did not observed significant differences to condition index relative to the control and multiples comparisons. In the other hand, Dunnett and Tukey test identified significantly differences between control and multiple comparisons, the last one verified differences between 10°C and 25°C. Dunnett and Kruskal-Wallis test detected a significant differences between 20°C and 25°C (H = 10.89, df = 4, p = 0.028). It was improved to Student Newman Keuls test that was verified significantly differences for 10°C vs 25°C; 10°C vs 15°C; 20°C vs 25°C; 20°C vs 15°C; 30°C vs 25°C and 30°C vs 15°C (H = 10.89, Df = 4, p = 0.028).

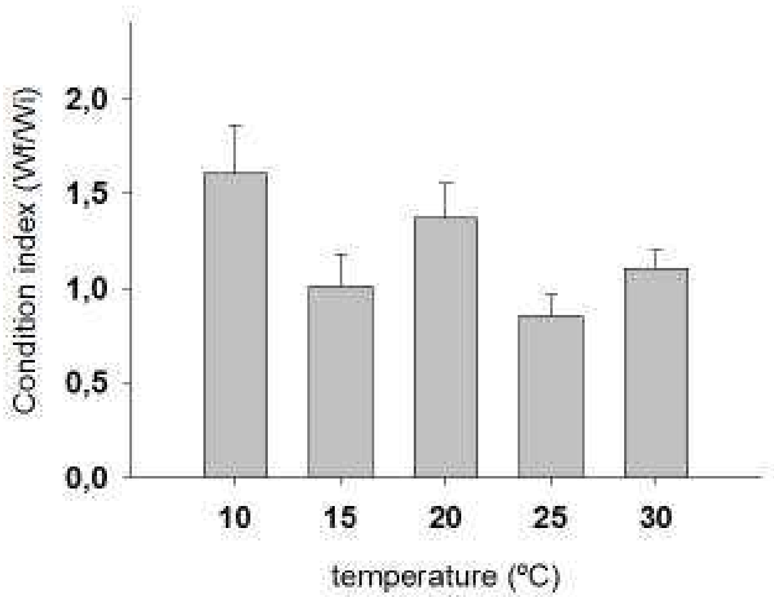


Figure 5. Condition index of sea anemones for tolerance test on each temperature. * Wf and Wi means final weight and initial weight respectively.

3- Behavioral parameters

The variation of the behavioral parameters tentacle flexion, tentacle retraction, oral disc flexion, column cavitation and peristome depression during the test period for each temperature depicted in Figure 6.

a. Tentacle Flexion

Tentacle flexion (Fig. 6A) was significantly affected by temperature (p = 0.012) and time (p = 0.011). Spearman’s correlation showed a significant correlation between tentacle flexion and temperature/time (p = 0.05). After 24 h of exposure the highest percentage of flected tentacles found at 15ºC (55%); the highest percentage of semi-flected tentacles found at 10ºC (60%) and the highest percentage of extended tentacles found at 25ºC (45%). After 96 h of exposure to the test temperature, the results were similar, except that the highest percentage of flected tentacles found at 20º (80%).

b. Tentacle Retraction

Tentacle retraction (Fig. 6B) was significantly affected by temperature (p=0.033). The higher percentages of tentacle retraction flected was found on 20ºC and 25ºC, with the same values (45%); semi-flected on 10ºC and 30ºC, with the same values (60%); extended on 15ºC (55%). On the last day, the higher percentages of tentacle retraction flected was on 25ºC (45%), semi-flected on 10ºC (35%) and extended on 20ºC (80%).

c. Oral disc flexion

Kruskal-wallis results it was observed that rejected the nulli hypothesis, between temperature and time variations (p = 0.008 and 0, respectively). It was done Spearman`s correlation and observed that exist correlation between oral disc flexion and only time (δ p = 0.05). On the first day, it was higher percentages of oral disc flexion flected on 20ºC (35%), semi-flected on 15ºC (80%) and extended 25ºC (40%) (Fig. 6C). On the last day, it was higher percentages of oral disc flexion flected on 20ºC (80%), with a 45% of increment in comparision to the first day. Semi-flected higher percentages on 10ºC (40%) and extended on 25ºC (45%), with only 5% of increment in relation to the first day (Figure 6C).

d. Column cavitation

Kruskal-wallis results it was a significant differences between temperature variations (p = 0.052). It was done Spearman`s correlation and observed that exist correlation between tentacle flexion and only temperature (δ = 1). Post-hoc test observed significant differences on column cavitation openness/close on 10°C and 30°C data (p = 0.02) (Fig 6D). On the first day, it was higher percentages of column cavitation flected on 20ºC (40%), semi-flected higher percentages on 25ºC and 30ºC, with the same values (85%). The extended on 10ºC and 15ºC, with the same values (20%). On the last day, the column cavitation flected with higher percentages was on 10ºC (20%), with a decrease of 20ºC of 30%. Semi-flected higher values 15ºC (95%), almost the totally of individuals, with a decrease of 30ºC (35%). The column cavitation extended higher percentages was on 20ºC (85%), with a decrease of 10ºC and 15ºC to nuli.

e. Peristome depression

Kruskal-wallis results it was observed that rejected the nulli hypothesis, between temperature and time variations (p = 0.025 and 0.018, respectively). It was done Spearman`s correlation and observed that exist correlation between oral disc flexion and only time (p = 0.008) (Fig 6E). On the first day, the higher percentages of peristome depression flected was found on 10ºC, 25ºC and 30ºC (15%), semi-flected on 25ºC and 30ºC (85%) and extended on 10ºC (20%). On the last day, the higher percentages of peristome depression flected was on 10ºC (20%), with a 5% of increment in comparision to the first day. The semi-flected on 20ºC (90%), almost the totally of individuals, with a decrease of 30ºC of 35%. The extended on 30ºC (40%), with a decrease of 10ºC to nuli.

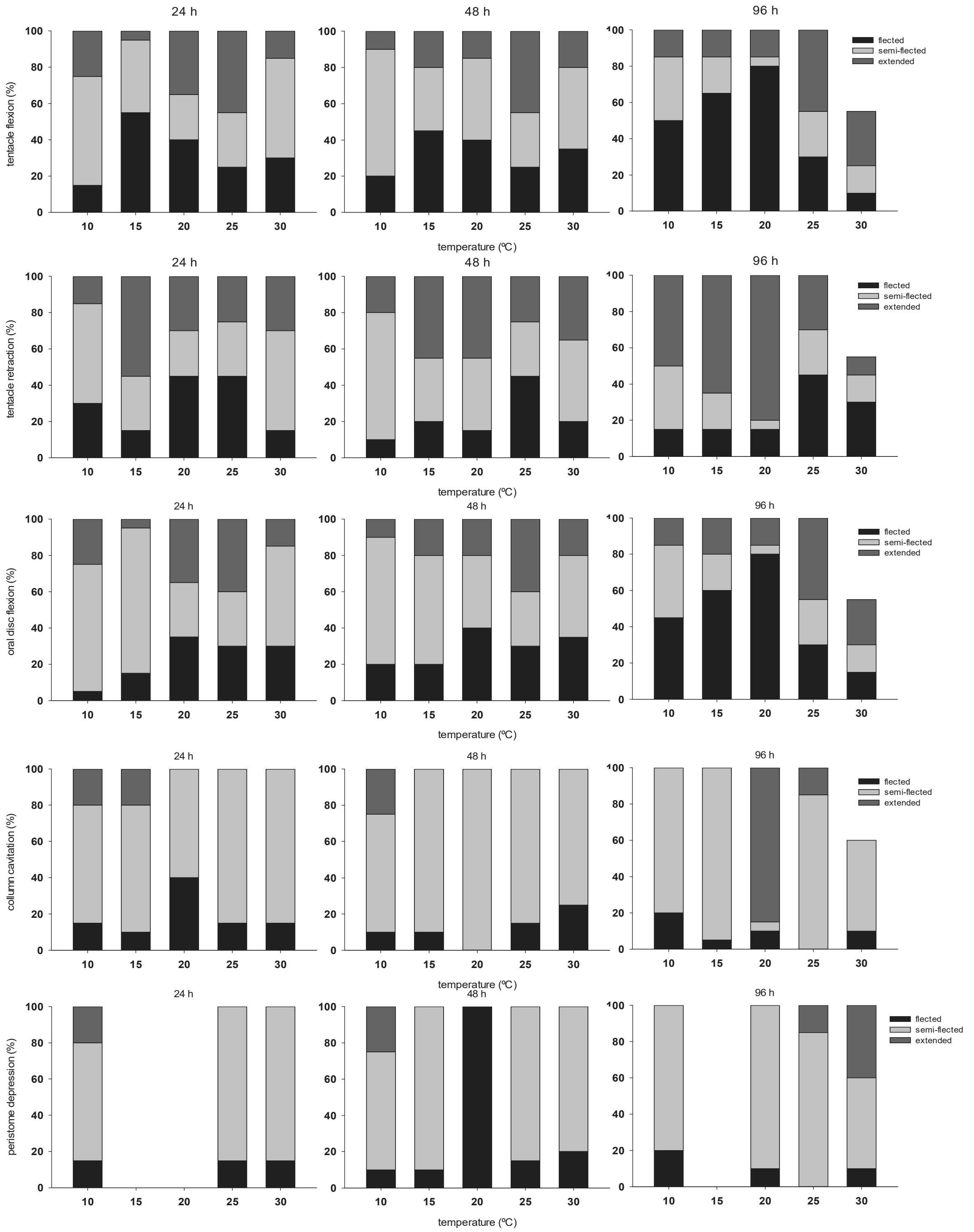


Figure 6. Behavior levels percentage variation with temperature. In a scale of flected, semi-flected and extended open/close of each behavioral parameters. A- Tentacle Flexion; B- Tentacle Retraction; C- Oral Disc Flexion; D- Collumn Cavitation and E- Peristome Depression.

DISCUSSION

The main objective of this study was to assess the temperature tolerance of *Actinia equina* to temperatures between 10 and 30ºC, and assess their responses (condition index, reproduction and behavior) over time (96h). A wide range of temperature was test in order to establish a "worst case scenario".

The results showed that their anemones responses were significantly affect by temperature and varied during the exposure period. The results of this investigation show that transient low and high temperature stress in 96 hours causes a variation on behavior parameters in the populations of sea anemones *Actinia equina* and a series of chemical alterations on water. Maybe results of sea anemones metabolism alteration are due to temperature variation along time.

Before the test, anemones were maintain in a flow-through system under controlled conditions, receiving artificial fish diet feed, containing a low lipid level, but constituted of nutrients such as proteins, vitamins, fiber, ash and oil. During stress periods, sea anemones have ability to retain large quantities of food and water into the gastro vascular cavity. The uptake or release of food and other natural physiological products could explain the variations of nutrients and chlorophylls during the test (Chomsky *et al*., 2004a; unpub Gadelha *et al*, 2010).

In relation to survival, it was only reduce at 30°C test (almost 50% of the total number of organisms). This could be explain by the fact that this species is natural from temperate ecosystems, thus more tolerant to decreasing temperature than increasing temperature (Chomsky *et al*., 2004b).

Besides survival, also the reproduction of anemones was affect by temperature. In all temperature tolerance, tests it was observe increase on number of polyps in relation to the first day, with juvenile polyp's liberation, with a significant difference between 10°C and 25°C. The increased reproduction at extreme temperatures is probably due to the stress caused by temperature, leading to a reproduction investment under stress conditions, aiming to save the population. However, there could be a "temperature/time limit" to these stimuli.

The condition index was also affect by temperature, showed higher decrease at 10°C and 30°C. This could be explain by the fact that ranges near the ideal temperature are not enough to implement the loss weight. At 15°C and 25°C it was detected an increase on condition index. The present work agrees with a previous work studying with the species tolerance temperature, in the rocky intertidal zone of the Mediterranean coast of Israel, they examined variation in polyp growth at several temperatures within the local range (Chromsky et al., 2009). Under laboratory conditions, the authors observed that only polyps at low temperatures (15 and 20 °C) grew, whereas those at higher temperatures (25 and 30 °C) lost body mass (Chromsky et al., 2009). Another important conclusion it was that at summer seawater temperatures along the coast of Israel (28.7–29.5 8C), polyps of *A. equina* are unable to balance their metabolic requirements with energy input, resulting in a seasonal reduction in biomass. Polyps appear to be able to acclimate to high temperatures, but not sufficiently to avoid shrinkage of tissues (Chromsky et al., 2009).

The physical-chemical parameters were maintained constant along to 96 hours, although they showed statistically significantly differences among temperatures, but these variations are not ecologically relevant (according to USEPA, 1994).

The chlorophylls values not showed significantly differences between temperatures ranges. Temperature, leads to increased chlorophylls a and c levels. This research was in agree with Dunn et al (2004), who compared the chlorophylls a and c values in relation to temperature stress on water over time, not finding any significant differences. They showed that heat stress could initiate cell death pathways that differ in rate and magnitude. The rapid response contrasts with the effect of elevated sea-surface temperatures for reef ecosystems, which is currently determined in terms of degree heating weeks. In bleaching events in the field, relatively small diurnal fluctuations in temperature may be far more important than previously recognized and may selectively promote the rapid Programmed Cell Death response over slower necrotic cell death. The timing of the peak of apoptosis-like cell death, at around 3 h exposure, was similar in ectoderm and endoderm cells and at all temperatures, but was more pronounced (higher frequency) at higher temperatures.

Nutrients composition showed differences with temperatures variations over time. When compared to control (20ºC, supposed ideal temperature) the nutrients concentrations decreased with increasing temperature. It was maybe due to sea anemones metabolism increase/decrease. Higher consumption of oxygen and hypoxia condition on higher temperatures (30°C), elimination of nitrogen compounds on water, increasing the pH and consequently increasing the nutrients reactions and consumption. Many species of marine invertebrates, such as corals, sea anemones and giant clams, harbor endosymbiotic dinoflagellates (zooxanthellae) in their tissues. Zooxanthellae release short-term photosynthetic products to their hosts and so are an important source of organic carbon for host metabolism, growth and reproduction. Zooxanthellae are also important in the recycling and conservation of essential nutrients, such as nitrogen, and enhance calcification rates in corals (Muscatine 1991).

Thermal stress in laboratorial climate change simulation could to induce other effects on these species of sea anemones. A recently study, to conclude that the high levels of genetic polymorphism and sexual reproduction (even though rarely) documented in Israeli populations of *A. equina* result from environmental stress experienced by members of this species at their southernmost limit of distribution in the Mediterranean region (Chomsky et al., 2009). Environmental stress known to increase genetic polymorphism, through high rates of mutation and recombination. This pattern extends to populations in Croatia, where applied the same AFLP methodology to *A. equina* living in much cooler water than in Israel, and revealed locus polymorphism only about half (36.8–47.2%) as high as that in Israel (56.1–73.5%). They conclude that the exclusively sexual reproduction, high genetic diversity, and low abundance of Mediterranean populations of *A. equina* all may be cause in part by the stressful environmental conditions experienced by members of this species in this region. Under the less stressful environmental conditions in northern Europe, asexually produced offspring may form large aggregations that perpetuate parental genotypes, which are well adapted to the locally benign conditions of low temperature for this species. Sexual reproduction is thought to enhance the long-term success of organisms, since it generates a wide range of genotype escapable of "tracking" environmental changes (Chromsky et al., 2009; Chromsky et al., 2004 a,b,c).

Beitinger and McCauley (1990) suggested that responses to environmental changes could be divide in four categories: passive – no response, when the stimulus is not sense or occurs too rapidly thus leading to a decrease in performance capacities or even death. Behavioral reactions – when subjected to certain chemicals, animals usually react in seconds or minutes, avoiding stress and trying to obtain a favorable position relative to the level of stimulus. Physiological responses – organisms suffer internal changes in various physiological processes, including adjustments in physiological rate functions and tolerance acclimation enhancement, which may occur within hours to weeks. Finally, biochemical responses – synthesis of new molecules like ―stress ‖ proteins in response to environmental changes, in order to restore homeostasis within genetic constrains, which may take from days to weeks. Therefore, adding behavior as an endpoint can help to formulate a quantitative minute-to-minute or hour-to-hour assessment of how tested species are. Re-acting towards the toxicant concentration, bearing in mind that behavior can be classified as the cumulative interaction of a variety of biotic and abiotic factors that represents the animal's response to internal (physiological) and external (environmental, social) factors and that relates one organism to another (Dell'Omo, 2002). Behavior provides an insight into various levels of biological organization, being a result and determinant of molecular, physiological, and ecological aspects of toxicology (Scott and Sloman, 2004). Therefore, behavioral responses may reflect biochemical changes in the individual organism and subsequently promote alterations in communities, which can be translate into ecological consequences (Lagadic et al., 1994).

Behavioral parameters were significantly affect by temperature and varied over time. The ecological interpretation of each behavioral parameters could be translate as physiological functions. Tentacle flexion is a response to specific photoreceptors in that the maximum efficiency for stimulation is in the same spectral regions as for many forms with discrete photoreceptors. The analogous behavior parameters to this was tentacle retraction that is considered to be a responses to absorption of energy by proteins and nucleic acids, as evidenced by it is maximum efficiency peak at 280 nm. The results showed that tentacle flexion/retraction were also affect by temperature and time. A correlation between temperatures and time variation found, meaning that the tentacle expend a large time to absorptions energy in proteins and nucleic acids forms. The oral disc flexion, column cavitation and peristome depression responses involve regional muscle action, probably by deep photoreceptors rather than by nonspecific effects on cell proteins. This answers could be translated by timing expended to producing energy to accumulate, and how much temperature elevate how energy was expended and involving on cellular reactions. However, oral disc flexion and peristome depression only presented correlations in relation to time variation. In another hand, column cavitation only showed correlation in relation to temperature variation (extreme 10°C and 30°C). The column contraction could activated by seawater contact and the temperature could be the first stimuli to this act. Therefore, the species Actinia equina, spend a more time with retractions tentacles, because they did not has symbiotic algae to help in an increase against temperatures expositions protections. They could was involved a deep photoreceptors nonspecific to develop the cell protein production as a secondary function (Di Marco, 2008).

The most of bibliography focused on symbiotic relations and they anatomic and physiological thermal stress effects. So the present work, try to find a way to explanation these environmental and global changes effects on solely organisms, to generalize to all populations (Weiss, 2008; Richier et al., 2006; Lesser et al., 2006; Dunn et al., 2004; Fitt et al., 2001; Perez et al., 2001 and Gates et al., 1992).

The present research could be consider a first step to find baseline behavioral answers to environmental and climatic changes as early warning systems, using sea anemones as sentinel species. Because was tested such of behavioral (traduced as chemical effects) on thermal controlled induced effects along the short-time exposure. Behavior endpoints could be the first signals to physiological alterations and give us time to try to return or revert the first alterations and comprehend how we can proceed to explain the environmental and global alterations, focused on climatic changes.

Elevated temperatures and solar ultraviolet (UV) radiation been implicated as recent causes for the loss of symbiotic algae (i.e., bleaching) in corals and other invertebrates with photoautotrophic symbionts. Until, aposymbiotic organisms could reveal water physical alterations, in relation to increase of chlorophylls levels.

Clark and Kimeldorf (1971) studied behavioral reactions of the sea anemone, *Anthopleura xanthogrammica*, to ultraviolet and visible radiations. They test for first time, the behavioral parameters to use on the present study and they concluded ecological interpretation for each endpoint, such as tentacle flexion appears to be a response to specific photoreceptors in that the maximum efficiency for stimulation is in the same spectral region as for many forms with discrete photoreceptors. Tentacle retraction considered a response to absorption of energy by proteins and nucleic acids, as evidenced by its maximum efficiency peak at 280 nm. Oral disc, column cavitation, and peristome responses involve regional muscle action, probably induced by deep photoreceptors rather than by nonspecific effects on cell proteins. The early stress response to elevated temperature involves essentially all aspects of same chemical reactions; in this case, we observed a receptors functioning and the frequency of open-close oral sea anemones, tentacles and columns anatomic alterations to detect earlier the effects of physical stress induction.

The results of these study suggest that the behavior alterations reveal that short-therm effects of temperatures variations could not evoke substantial consequences, but on natural environment, the organism were expose to multiple stressors. *Intertidal organisms are subject to a variety of stresses such as desiccation, water temperature, acidification, increase salinity, nutrient limitation, space competition and predation.*

CONCLUSION

The present work showed that sea anemones are sensitive to environmental and global changes. The physical stressor (temperature) tested in the scope of a climatic and environmental changes simulation, showing that differences on sea anemones behavioral answers, demonstrated that these organisms have potential to use as early warning systems to climatic changes.

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CHAPTER 6.

Gereral Discussion, Conclusion and Future Work

Chapter 6. General Discussion

The main aims of this dissertation was to determine if natural populations studies, chemical and physical laboratorial stressors simulations could be provide enough information about marine and coastal environment state; and if congeneric sea anemones could be used as early warning to environmental studies and climatic changes predictions.

On the last chapters, it was identify selected cosmopolitan targer species to environmental evaluation, samples sites based on differents pollution sources, based on previous studies, it was selected chemical to simulating more realistic environmental scenarios. This will provide a better understanding and prediction of adverse effects of chemical and physical stressors on natural populations of sea anemones. Additionally, it was created an environmental data basis of water physico-chemical composition, chemical assessment and enzymatic on sea anemones tissues and sediment from three importants climatic scenarios. Finally, behavioral data of temperature stress tolerance, it was created in order to better understand one of the most relevant of climate changes issues on the recent times: the global temperature oscilation.

The last decades the climate changes it was an importante discussion topics. Many researches, investigate the global and environmental changes focusing on marine and coastal environments. Over the centuries, the populations migrate on direction of the litoranean zones, trying to stablish a good relation between life quality vs comercial exploitations (fishering, Harbour facilities, comercial relations between countries, etc). Due to this fact, these coastal zones receive an increment of antropogenic residues: industries (toxic chemical residues), domestic garbage (personal care products and organic material), agricultural (carry out the pesticides and herbices to the rivers and sea) and pharmaceutical compounds (estrogenics substances). The global populations increase of a great proportions that the environment could process all the impacts. These human being manipulation of the environment cause a great stress to Earth and provoque a such phenomena of global warm. Coastal areas are highly sensitive against climate dependence and each region is affected by the impacts of climate shifts representing different degrees of vulnerability, climatic phenomena that control dependence of the El Nino Southern Oscillation (ENSO) and high tropical North Atlantic (TNA) Sea surface temperatures (SST). Changes in ocean circulation can affect the regional circulation of shelf and coastal seas, leading either to increase export of nutrients plus carbon from the shallow seas into the open ocean or to increased upwelling of nutrients plus carbon onto the shelf and towards coastal areas (Walsh, 1991; Smith and Hollibaugh, 1993; Borges *et al*., 2005). Temperature, salinity and precipitation regimes as well as a wide range of several physical variables due to tidal cycles and freshwater runoff from land put additional pressures and drive changes in coastal ecosystems (Milliman & Farnsworth. 2011). The present study obtained results that the three main environment types (tropical,subtropical and temperate) identified different patterns of water conditions, latitudinal differences and variety stress sources, and the influence of the climatic patterns of the physic-chemical variations, mainly temperature and salinity, on the other factors in each climatic zone (Gadelha et al, 2015 a) (Chapter 2) (Figure 4, 5 and 6). The mechanisms of organic matter and nutrient relationships, such as a natural disturbance leading to resuspension, need further research to discriminate the impacts of increasing anthropogenic inputs on biogeochemical dynamic. Overall, despite the relatively high environmental and anthropogenic influences in the studied marine coastal areas, effective processing of different sources and forms of nutrients and organic matter ocurred and significantly reduces their signatures. Most of these materials were largely transformed and decreased in amount probably because of flocculation and removal to sediment, microbial degradation and/or dilution by other organic matter sources prior to export to the coastal ocean (Borges *et al*., 2005; Milliman & Farnsworth. 2011; Valiela, 1995; Marengo *et al*.. 2005; 2011, 2012).

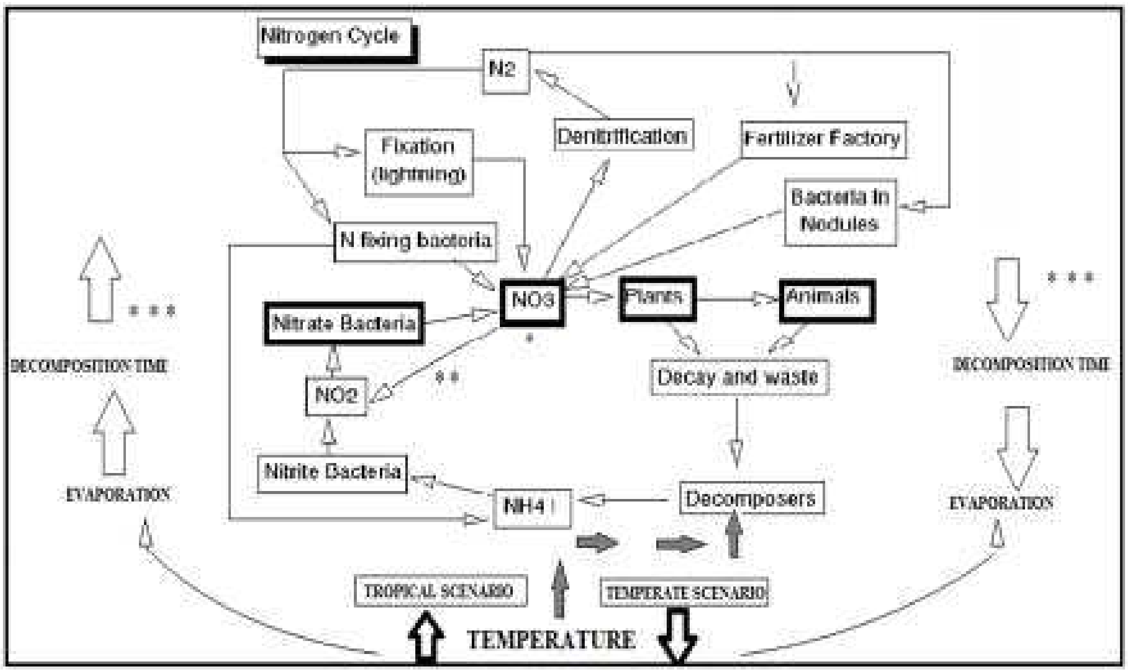

Figure 3. The transfer of nitrogen in environment. The boxes are pools while the arrows represent processes. The negrit boxes meaning the mainly routes of absorption of nitrates, with the processes with asteristic (*): the most oxidizing of nitrogen Nitrates (NO^{3-}); represented with double asteristics (**): the reduction reaction of nitrates to nitrites (NO^{2-}), denitrification processes require a supply of organic compounds. Represent with three asteristic (***) and gray arrows show that the temperature dependence on Ammonium formation by decomposer, interfering on the decomposition time (Adapted by Valiela, 1995).

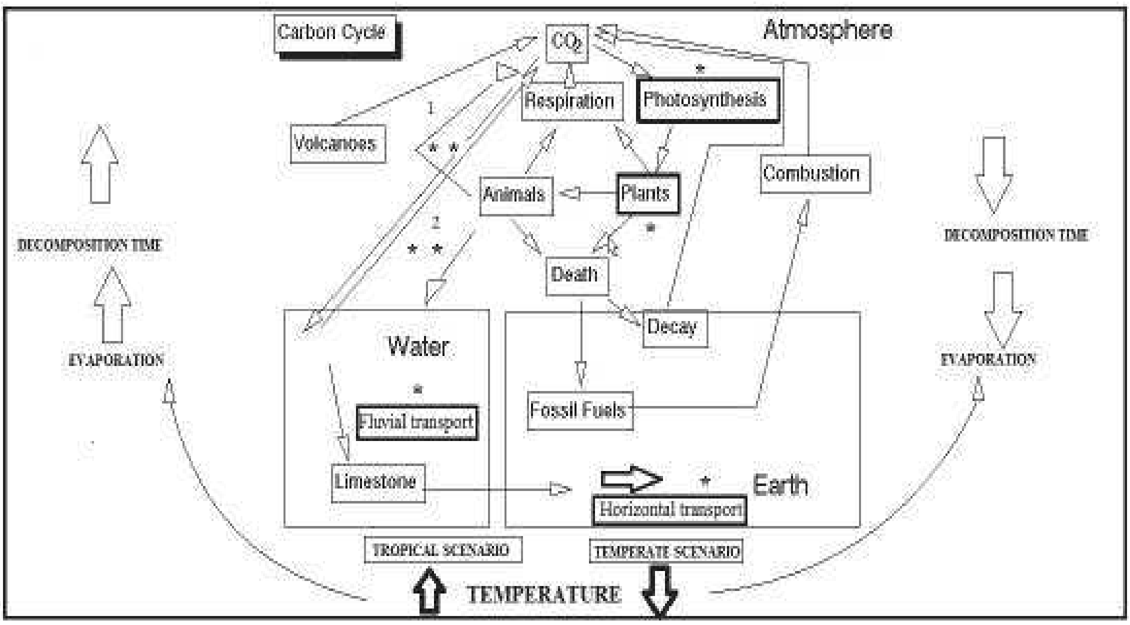

Figure 4. The transfer of carbon in environment. The boxes are pools while the arrows represent processes. The negrit boxes meaning the mainly external factors that contribute to DOC formation, with the processes with asteristic (*): Photosynthesis, vascular plant POC transport carried by rivers; fluvial and groundwater transport and horizontal transport of terrestrial estuarine and coastal organic matter to deeper sediment; internal factors represented with double asteristics (**): 1- excretion and 2- metabolic waste secretion (Adapted by Valiela, 1995).

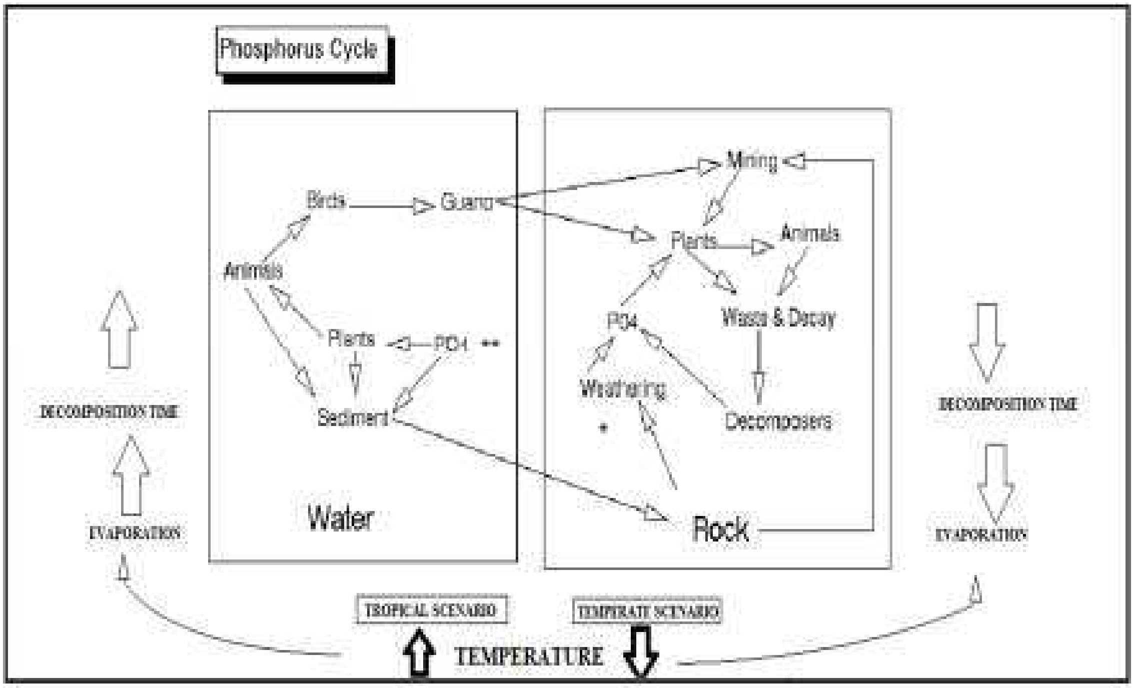

Figure 5. The transfer of Phosphorus in environment. The boxes are pools while the arrows represent processes. Marked with an asteristic (*) is the most important factor involved on phosphates transformations on the cycle. Because of the chemistry of the phosphorus, it is therefore clear that, at least for human use, phosphorus must be treated as a nonrenewable resource in limited supply. With double asteristic (**) show that Regeneration of phosphates is temperature dependent (Adapted by Valiela, 1995).

Ecotoxicology is a Science that study all the environmental alterations and effects using a certain vehicles, called biomonitors or bioindicators. The biomonitors could be any organism existing in environment, since them receive the environmental stimuli. But they should to respond to a few criteria: 1- to be resistant, 2- easy handling, 3- reproducible, 4- sensitive. In the last decades, ecotoxicology improve a large number of bioindicators, such as gastropoda, crustacea, fishes, collembola, anellida, bivalve, echinodermas, the most recent birds, amphibeans and reptiles. Finally, the present study introduce a cnidarian representant: the sea anemones (Vaufleury, 2000; Lange et al., 2006; Lee et al., 2009; Nagel, 2001; Fountain and Hopkin, 2005; Lowe, C. N., & Butt, K. R. 2007; Lowe and Butt, 2007; Farris and Van Hassel, 2006; Paredes and Bellas, 2009; Koivula and Eeva, 2010; Boone and James, 2005; Amaral et al., 2012; Gadelha et al., 2013). The majority ecotoxicological studies it was developed under laboratorial controlled conditions. These fator could be a limitant, in order to realistic extrapolations of the obtained results. Because, these kind of tests, do not predict and simulate the external fluctuations factors, such:

water flow, tides, human bein manipulations and populations interactions. The presente study, tried to find a way to reproduce one of the most relevant factor of climatic changes: the temperature (Chapter 5). The first step to conduct these work was to characterize physical, ecological and chemically the study areas (Chapter 2). After these step, to quantify the present pollutants (bioaccumulation) and to test pollutant effects (biomarkers) (Chapter 3 and 4, respectivelly).

Our results on paper 1 were developed in three different climatic regions, emphasizing the important contribution to the climate knowledge and temperature increase/decrease interferences about dissolved oxygen, dissolved organic carbon, total dissolved carbon, pH, nutrients and suspended particulate material on "natural"dynamics.

Tropical coastal areas usually present several differences from temperate coastal areas, including the higher rainfall rates, the presence of perennial vegetation that reduces the soil erosion rates and a limited temperature variation. Besides that, the rivers from these regions present high water discharge, transport a smaller amount of suspended particulate matter (mainly due to the vegetation presents a higher density) and present a higher nutrient concentration (Meybeck, 1982; Nittrouer *et al*, 1995; Zhang, 1999). In temperate areas, physicochemical and biotic conditions usually display clear temperature dependent seasonal patterns (Drake and Arias 1991; Childers *et al*. 2006 a).

Temperature and salinity are important climatic parameters due to its influence in the suspended particle aggregation and water quality (Gleick, 2003). The patterns of temperature variation in this study were the most expected and predictable, due to the location of sampling sites in three different climatic regions, and climate has a direct influence on water temperature, mainly determined by the air temperature (Sarmiento *et al*, 2004, Steinacher *et al*, 2010). Salinity variations are also important due to its influence on the flocculation/ aggregation, and hence the settling velocity, of the suspended particles (SPM), the higher the salinity, the greater the aggregation of suspended particles, the bigger the flocs and the faster the settling velocity (Syvitski & Milliman, 2007, Milliman & Farnsworth. 2011). The low salinity values relate to the proximity to estuarine mixing zones, because the diurnal evolution of salinity and temperature generally varied with tidal influence. Warmer water tends to have lower levels of dissolved oxygen. Low water velocities promote lower turbulence which results in lower dissolved oxygen with effects on water quality. In these conditions, the BOD and NH4+will increase while the dissolved oxygen will decrease (Mimikou *et al*., 2000).

Primary production is strongly limited by light availability and suspended particulate matter (SPM). This mainly affects photosynthesis and water temperature. Under non-nutrient limiting conditions, light availability is often the predominant factor in determining Chl *a* variation in coastal waters (Pennock, 1985). The last two phenomena are directly linked to climate change (Guo *et al*. 2009). Other factors tested such as nitrification rate is a temperature dependent process, corresponding to higher temperatures to lower nitrate concentrations. However, this might be overwhelmed by the changes in the dilution and residence time associated with changes in river discharge (Walling & Webb, 1992; Arnell,1998; Mimikou *et al*., 2000). The hipoxia phenomena fouded on samples from temperate and tropical could be explained because the collect it was done on low tide, so the oxygen levels were affected by the internal or external factors relating the nutrient cycling's.

The "difuse" sources of organic material (agricultural, human activities, soils, topography, land uses), could interfere on Organic carbon losses resultant from the microbial decomposition processes, in anoxic environments, increase nitrites, nitrates and sulfates inputs (Valiela, 1995) and in nitrogen cycles dominated by a gaseous phase and microbial transformation involving changes in the oxidation state cause by denitrification, consisting of NO3- transformation to NO2- and requires a supply of organic compounds. Polyphosphates traces is a natural component of seawater and the high concentration often could used as a pollutants indicators. The phosphates significant differences obtained in the present work might be due to the spatial variations (distinct climatic zones). Some studies suggested that phosphorous regeneration has a strong relation with bathymetry, where in coastal areas the renewable is fewer than 70%, less than in oceanic waters that could be near of 99%. Oxygen uptake by organisms in water correlates to phosphorus (and nitrogen) concentrations, since nutrients are released during aerobic respiration of organic matter (Valiela, 1995).

On the other hand, tidal influence is also very important not only for hydrographic parameters but also for dissolved and particulate organic carbon, nutrients and suspended particulate matter, because tides have substantial effects on carbon sequestration (Ribas-Ribas *et al*., 2011a) and low tides anoxic conditions could contribute to DOC low levels (Gwenaël *et al*, 1999). The high values of POC and DOC obtained in this work showed the importance of the strong influence of estuarine proximity and river discharges, industrial effluents, agricultural runoff, and domestic sewage discharged into the surface waters, in each sampling sites in the three climatic regions, resulting in increased levels of POC and DOC.

The coastal areas physic-chemical characterization and carbon, nitrogen and phosphates variations in three climatic scenarios described here reveal a large input of nutrients, POC, DOC and Chlorophyll *a*., compared with other studies reported in the Atlantic and continental water (Smith and Hollibaugh, 1993; Bianchi *et al*, 1993; Alongi, 1995; Ganeshram et, al, 1995, Gattuso *et al*, 1998, Biondi *et al*, 2001, Brunskill, 2009, d'Orgeville & Peltier, 2007, Passow & Carlson. 2012). The present work investigates some correlations between factors, such as temperature influence (spatial variation) in other physic chemical factors, and also negative values were found in tropical and subtropical sample data. This is due to the sampling sites situated in strictly estuarine zones correlation with marine sampling locations, receiving the freshwater influence at low tide collections. The selected water parameters were able to discriminate the three climate areas. These data can then be used in ecosystem level models to predict the effects of climate change on marine and coastal habitats and potentially to evaluate the effects of different management scenarios.

The main objectives of the paper 2, it was studied the presence of contaminants on congeners natural populations and quantify de bioaccumulation levels on tissues and sediments in three diferente zones.

The results showed that exist an spatial variation on chemical bioaccumulations levels, according to the Shiedeck et al (2007) models. This reference suggest that the POP`s have a tendency to disperse latitudinally, since low latitudes on direction to high latitudes. The contaminant range direction has a strong tendency to follow the tropical to temperate latitudes (hot to cool latitudinal site). In low latitudinal places, the tendency is to high evaporation and low deposition, in opposite to this, the high latitudes, the depositions levels is higher than evaporation. The bioaccumulations levels vary with latitudinal localization and between species too.This intraespecific variations it due to the ecological niches of these species, is very importante to distinguish until the effect observed it is caused by stressor, because the one of they, could be linked to the representant of symbiotic organisms (zooxanthellae).

Answer to a question that if sea anemones could be a good tool to environment hazard, it due to, in general, benthic invertebrates are reported to be ideal assessment indicators because they are relatively non-mobile and thus tend to be representative of the area being sampled (Reynoldson et al., 1995 in Dolenec *et al*, 2007). The use of sea anemones derived from the fact that this species showed a large distribution. In other hand, they are known as nonselective suspension feeders that hosting abundant bacterial populations, which may have differential contaminants values, and high bioaccumulation values, that could show a better description of the overall impact on the environment due to the presence of enriched sources and many types of discharges.

The last question remain about the Chapter 3, is: if is possible to create a geographical pattern of POPs distribution. Our results showed that, yes, the sea anemones contaminants bioconcentrations present spatial and interespecific variation. In general, sea anemones could show the highest concentrations of persistente organic pollutants (POPs), due to filter feeding, the lack of sophisticated detoxification pathways and theirliving inside the sediment. The intraspecific variations being indicative of sea anemones as selective bioaccumulation indicators, possessing morphological and physiological adaptations to environmental forcing factors, which include eventual pollutants. This could be due to a combination of differential species susceptibility to biotransformation and variation in kinetics due to different physical– chemical properties (Denton and Burdon-Jones, 1986; Hanna and Muir, 1990). Comparing the type of samples used on these study (sediment and sea anamones tissues): the results reveal that exist higher concentrations of HPAs on tissues than on the sediment, in oppositive POPs showed a higher concentration on sediment. These chemical contaminants PAHs, CBs and chlorinated pesticides are known to be usually strongly associated with sediment, while being almost absent in the water phase. They mainly attach to the fine fraction while coarse particles present only few active spots. A knowledge of the trends and dynamics of OCs and PAHs between sediment and marine invertebrates is important for the understanding of trends and patterns of contaminants overall in marine ecosystems. For chemical analysis, species of interest must be limited in mobility, sufficiently present in the catch, important in the ecosystem and potential accumulators of pollutants. Pollutants uptake occurs problably via ingestion of contaminated food, and accumulation and depuration processes, can occur during sea anemone life cycle depending on the diet, water conditions and individual susceptibility for environmental contaminants. These organisms may have the ability to accumulate large quantities of PAHs and POPs compounds from the environment, and also plays an important role in the storage, redistribution and detoxification (Lobban & Harrison, 1997; Harland et al., 1990a; Hurd,2000). These results confirm the importance of filter feeding organisms, such as sea anemones, potential for pollutants bioaccumulation, coupled with the slow rate of excretion and metabolism, leads to biomagnification, from marine sediments to the food chain (Mason et al., 2006).

Following the European directives guidelines of the Water Framework Directive and Marine Strategy Framework Directive, this study has provided data on the PAHs and POPs concentrations found on sediments, sea anemones tissues and in selected sea anemones species, in order to provide the evaluation of ecological and environmental status of the three different climatic coastal areas.

The article 3 investigate another way of environmental chemical pollutants contribution to damages and effects on natural populations.

Our results, support that enzimatic activity on natural populations show a significantly diferences between climatic scenarios. The results reveal that exist a difference on enzymatic answer according to the pollutants sources where came from each species. Concluding that exist a spatial (latitudinal) and interespecific enzymatic differences. Is possible to stablish a scientific biomonitoring programe based on marine systems enzymatic answers using natural populations from three climatic scenarios. In general the organisms from contaminated stations exhibited significantly lower mean enzymes activity levels than in reference stations. *A. equina* and *A. sulcata* contributed to a better differentiation between reference and contaminated areas. It has been described that clearly differences between symbiotic and aposymbiotic anemones related to its responses to contamination, due to possible differential contaminants accumulation by the algal symbionts (Mitchelmore et al. 2002). A common mechanism employed to exclude and control contaminants entry is the binding of contaminants by external mucus coatings, symbiotic anemones appeared to produce more mucus than aposymbiotic ones (Merle et al. 2007). Studies investigating the bleaching phenomenon and symbiotic relationships in corals use *A. sulcata* and *A. equina* as models (Richier et al. 2006; Merle et al. 2007).

The studied sea anemones revealed a number of properties that make them useful sentinels for environmental monitoring and can provide a measure of environmental pollution. Biochemical biomarkers, including antioxidant enzymes and evidence of oxidative damage to biomolecules, are powerful tools for detecting the exposure and biological effects of pollutants, allowing early detection of environmental problems and may reveal biological effects induced by pollutants under the influence of different climatic marine scenarios (Regoli et al., 2002a,b). Sea anemones are among the most abundant representatives and cosmopolitan species of coastal benthic communities (ca 6000 species) inhabit virtually all marine environments. They are abundant, ecologically diverse and can be found at different regions, included in contaminated areas (Rodriguez et al. 2007). These cnidarians are present on rocks and other hard substrates in shallow coastal water and like other soft-bodied marine invertebrates (symbiotic relationship) so far studied are able to utilize organic substances dissolved in the sea for their metabolism.

Overall results suggest SOD as the main discriminant biomarker, followed by CAT and LPO. The selected biomarker responses, observed in organisms captured along the three climate zones, indicate that *A. sargassensis*, *A. bermudensis* and *A. equina* revealed to be the most sensitive organisms in the tropical, subtropical and temperate areas, respectively.

A. equina is an intertidal specialist that reaches its highest densities on wavecut platforms on the lower portion of shores. The intertidal habit is subject to waves and surge, the water being laden with sand, stones and debris, as well as containing planktonic, nektonic and benthonic animals that can be dashed against the rocks. *Actinia* sp. diet is heavily influenced by shore height and wave exposure, but also that *A. equina* scavenges (rather than preys) on the larger food items that it ingests were known to take up dissolved organic material, to assimilate energy from bacteria and microalgae (van Praet, 1985), and to predate on a range of organisms (e.g. Chintiroglou and Koukouras, 1992, Kruger and Griffiths, 1998). *A. sulcata* is an example of a cnidarian that has strong regenerative abilities and employs asymbiotic relationship with photosynthetic zooxanthellae. It is commonly found on lower shorelines and sea beds in temperate regions (Muller-Parker and Davy, 2001). Green and brown colour morphs of this species exist and it is thought that the colour difference may be due to the presence of green fluorescent protein and pink chromoproteins.

Organisms respond to environmental changes by regulating metabolic pathways to prevent physiological damage. The physiological performance which reflects their evolutional adaptation to local environment (Dong et al. 2011; Somero, 2012), provide a direct link between thermal physiology and animals ecology, which elucidates the role of aerobic metabolism in the thermal tolerance across latitudes (Somero, 2012).

The results showed that the selected sea anemones species, directly exposed, at different latitudes and ecoclimatic regions, to extreme environmental conditions, presented consistent oxidative stress responses related with physiology alterations. The individual expressions precede population-level changes and are useful indicators if linked to specific physiological or ecological events (Dong et al. 2011). The degree of response seems to differ among species in relation to trophic level that they occupy, their habitat type, feeding habits, biotransformation capabilities and abiotic factors (Lee and Anderson, 2005; Barreira et al. 2007). Their habitats includes environments which are continually exposed to garbage dumping, untreated sewage inflow, land and river runoff, atmospheric fallout from heavy traffic and various small-scale industries scattered in coastal areas.

The results of sea anemones enzymatic responses approach in detecting differences among contaminated sites as the advantage of adopting lower taxonomic discrimination in the determination of perturbation in benthic communities. This appealing for cost and thus management reasons, and may thus reach a compromise between management and scientific objectives in the selection of appropriate species to discriminate in impact assessment programs.

The last paper 4, investigate the temperature tolerance capacity on behaviour of sea anemone *Actinia equina*. Behavioral parameters tested in order to answer the physiological consequences of temperature stress effects. The mainly question to answer was if behavioral answer to physical stressors could be signalizing an early warning to environmental and global changes. Combining behavioral parameters and changes on increase or decrease on nutrients, chlorophylls and physico-chemical parameters in relation to temperatures and time variations (sublethal test).

Survival, reproduction (juveniles polyps' liberation) and condition index was increase or decrease in relation to control temperature on temperatures/time variation. Behavioral patterns analysis placed the differentially ecological functions in a wide range of categories including tentacle flexion, tentacle retraction, column cavitation, peristome depression and oral disc flexion. This suggests that the early stress response to elevated temperature involve essentially all aspects of same chemical reactions, in this case we observed an receptors functioning and the frequency of open-close oral sea anemones, tentacles and columns anatomic alterations to detect earlier the effects of physical stress induction. The results obtained was that exist significantly differences on behavior with relation a temperature tolerance exposition along of time.

The results of this investigation show that transient low and high temperature stress in 96 hours causes a variation on behavior parameters in the populations of sea anemones *Actinia equina* and a series of chemical alterations on water.

The sea anemones have a strong potential to retain a large quantities of food and water during stress periods into the gastro vascular cavity. This fact could were explained the nutrients and chlorophylls increase/decrease during the test and more natural physiological products liberation in static water system (Chomsky *et al*., 2004; unpub Gadelha *et al*, 2010; 2012;2013).

It was observed a higher losses on survival at treatment to 30ºC (more than 50%), these fact could be related to the fasto f these species are naturally founded on temperate zones and tolerate better an decrease of temperature than de increase (Chomsky *et al*., 2004b). In all treatment, were a increase on juvenile polyps liberation, explained to the fact that these species invest on these strategy in order to garantee the species perpetuations, under adverse

cconditions. The results suggest that exist a "time limit" to increase/ decrease a condition index, detected increase at 15ºC and 25ºC and decrease at 10ºC and 30ºC (Chromsky et al., 2009).
The chlorophylls levels did not showed significantly diferences, but revealled a relation to increase according to temperature increment. The nutrients increase on the same direction that the temperature decrease. It was maybe due to sea anemones metabolism increase/decrease, higher consumption of oxygen and hypoxia condition on higher temperatures (30°C), elimination of nitrogen compounds on water, increasing the pH and consequently increase of the nutrients reactions and consumption.

Behaviour provides an insight into various levels of biological organization, being a result and determinant of molecular, physiological, and ecological aspects of toxicology (Scott and Sloman, 2004). Therefore, behavioral responses may reflect biochemical changes in the individual organism and subsequently promote alterations in communities, which can be translated into ecological consequences (Lagadic et al., 1994). Behavioral parameters showed significantly differences between temperature test tolerance and time variation.

The first parameters tested, tentacle flexion as a response to specific photoreceptors in that the maximum efficiency for stimulation is in the same spectral regions as for many forms with discrete photoreceptors. The analogous behavior parameters to this was tentacle retraction that is considered to be a responses to absorption of energy by proteins and nucleic acids, as evidenced by it is maximum efficiency peak at 280 nm. The results showed tentacle flexion/retraction rejected the nulli hypothesis, but showed a correlation between temperatures and time variation, this is mining that the tentacle expend a large time to absorptions energy in proteins and nucleic acids forms. The oral disc flexion, column cavitation and peristome depression responses involve regional muscle action, probably by deep photoreceptors rather than by nonspecific effects on cell proteins. This answers could be translated by timing expended to producing energy to accumulate, and how much temperature elevate how energy was expended and involving on cellular reactions. But, oral disc flexion and peristome depression only presented correlations in relation to time variation. In another hand, column cavitation only showed correlation in relation to temperature variation (extreme 10°C and 30°C). The column contraction, could be activate by sea water contact and the temperature could be the first stimuli to this act.
The most of bibliography focused on symbiotic relations and they anatomic and physiological thermal stress effects. So the present work, try to find a way to explanation these environmental and global changes effects on solely organisms, to generalize to all populations (Weiss, 2008; Richier et al., 2006; Lesser et al., 2006; Dunn et al., 2004; Fitt et al., 2001; Perez et al., 2001 and Gates et al., 1992).
Behaviors endpoints could be the first signals to physiological alterations and give us time to try return or regress the first alterations and comprehend how we can proceed to explain the environmental and global alterations, focused on climatic changes.

6.2. General Conclusion

The present work, used such of tools in order to assess the best environmental quality status and to find a good sentinel organisms to answer some climate changes questions. The coastal areas status is now best signilizaded for chemical and physical effects by sea anemones environmental and laboratorial studies. Sea anemones demonstrated was be a good early warning to environmental changes.

6.3. Future Work

For the future, the environmental and laboratorial studies using sea anemones or other symbiotic organisms, could be involve more easily and directly tools (Standard protocols) in order to save resources and decrease de time-answer for environmental simulations answer, with a most directly tools, after these results and conclusions; and could be developed a "package" of environmental hazard with benthic sentinel species. Maybe it will possible to apply the sea anemones protocols to other cnidarian, such as octocorals, hexacorals, Scleractinians (hard coral), softcorals and hydrozoarians.

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